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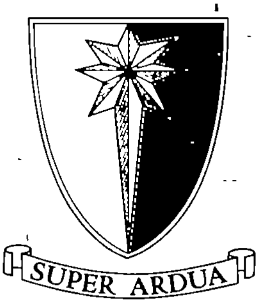

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MENTARY

The treatment of vaginal intraepithelial neoplasia after hysterectomy

aginal intraepithelial neoplasia (VAIN) are ally located in the vaginal vault and are found st often in women who have had a hysterec[illegible]my. In such patients this is probably a largely oidable problem; many of these patients were own to have CIN and were not examined colposcopically before their hysterectomies. At the very least a Schiller's test could have indicated the extent of the disease in the vagina.

After a hysterectomy which has been performed in the presence of unsuspected VAIN, abnormal epithelium is likely to be buried behind the sutures used to close the vault. Consequently a portion of the lesion will remain invisible and unassessable. In the series reported by Ireland & Monaghan in this issue of the Journal (pp. 973–975), 28% of those treated surgically proved to have unexpected invasive disease.

Before the possibility of buried disease was appreciated, the carbon dioxide laser was used to treat VAIN arising after hysterectomy. With increasing experience it became apparent that this was not an effective method of treatment. Of particular concern was the development of invasive disease in two of 14 such patients reported by Woodman *et al.* (1984). Thus, although the laser may be useful in reducing the size of a lesion or in treating women who have not had a hysterectomy, it should not be used as the sole method of treatment for VAIN of the vaginal vault after hysterectomy; the same caveat should apply to the use of topical 5-fluorouracil.

Surgical excision of VAIN gives far more satisfactory results (Lenehan *et al.* 1986; Ireland & Monaghan 1988). The patients in the series reported by Ireland & Monaghan (pp. 973–975, this issue) were treated by an abdominal operation or by a combined abdominal and vaginal approach. This necessitates mobilization of the ureters and separation of both bladder and rectum from the vagina. The alternative, vaginal approach avoids the need for such extensive dissection. However, when excising only the vault of the vagina in this way it is virtually impossible to clamp the vaginal arteries at the angles of the vault before dividing them. A brief period of brisk blood loss must therefore be anticipated. Whereas this procedure is often very straightforward, in patients with a narrow introitus and no laxity of the vaginal vault it can prove to be extremely taxing. Because only the upper 2–3 cm of the vagina need to be excised in most patients, postoperative coital problems are infrequent.

Where the lesion involves a large area of the vagina there may be a place for laser vaporization to reduce the size of the lesion. The entire lesion is vaporized under general anaesthesia and, some months later when the effect of the laser treatment can be assessed, residual disease may be excised. It must be emphasized that, even if no residual disease is apparent in the vault, it would be imprudent not to remove the top 1 cm of vagina. When this approach is unsuccessful and in older patients, total vaginectomy or radiotherapy may be called for.

There can be no doubt that intracavitary radiotherapy is a highly effective treatment for VAIN (Hernandez-Linares *et al.* 1980; Woodman *et al.* pp. 976–979, this issue). The two major concerns about this form of therapy are the possibility of radiation-induced cancer, and the effects it may have upon coital function. There have been several small studies in patients with carcinoma of the cervix which have raised anxieties about radiation-induced second cancers in the vaginal vault (Barrie & Brunschwig 1970; Futoran & Nolan 1976). While it seems possible that this may occur, it is probably an extraordinarily rare event (Choo & Anderson

1982; Boice *et al.* 1985). The dose of radiation required to eliminate a small focus of disease at the vault of the vagina is unlikely to cause major coital problems, especially if the patient and her spouse are encouraged to resume normal sexual relations as soon as possible (Woodman *et al.* this issue). Where the area of disease is more extensive, no method of therapy is likely to be free of the risk of inducing sexual dysfunction.

The management of VAIN after hysterectomy in a young woman is likely to be surgical. If the lesion is extensive, prior laser therapy may be helpful in reducing the extent of disease. In older patients, especially when access is difficult, surgery offers few additional benefits but carries a potential for greater morbidity. Radiotherapy is therefore likely to be the treatment of choice. Whatever the circumstances, it would be sensible for such patients to be investigated and treated by those with experience of this unusual but troublesome condition.

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Gynaecological disorders and risk factors in premenopausal women predisposing to osteoporosis. A review

S. Y. LAM, H. W. G. BAKER, E. SEEMAN, R. J. PEPPERELL

Osteoporosis is a common disorder with one-third of women over 65 years of age having vertebral fractures and by extreme old age, one-third of women having sustained a hip fracture (Riggs & Melton 1986*a*). Morbidity, mortality and costs of health care make osteoporosis a serious public health problem (Holbrook *et al.* 1984). The prevention of bone loss in perimenopausal women is an important aim in the prevention of fractures as there is no established safe and effective method of replacing bone already lost in elderly persons. There is no single cause of osteoporosis. Many diseases, as well as nutritional, social and environmental factors, influence bone mass in premenopausal women.

This review summarizes the effects of important gynaecological disorders and environmental factors that may affect bone mass of women both before and after the menopause. We emphasize the role these factors may play in predisposing to osteoporosis and fractures in later life. Osteoporosis secondary to medical disorders such as hyperthyroidism, hyperparathyroidism, Cushing's disease, rheumatoid arthritis and gastrectomy will not be considered.

Osteoporosis: definition and pathogenesis

Osteoporosis may be defined as a disorder characterized by decreased bone density (i.e. bone mass per unit volume) predisposing to fractures of the spine, distal forearm, and hip after minimal trauma (no more severe than that resulting from falling from a standing height). The term osteopenia is sometimes used to denote a decrease in bone mass, although strictly this term refers to the translucent appearance of bones on a standard radiograph. The presence of one or more fractures (non-traumatic or after minimal trauma) is used as a clinical definition to distinguish osteoporosis, the clinical entity, from radiological osteopenia or low bone mass as measured by bone densitometry.

Age-related and perimenopausal bone loss

Bone mass increases by growth and consolidation to attain a peak at the age of 30–35 years and then declines throughout life, at 0·3% per year in both men and women. Loss at cortical sites (appendicular or long bones) begins at about 45 years of age, whereas at trabecular sites it begins at least a decade earlier (Riggs & Melton 1986*a*). The perimenopausal acceleration of bone loss occurs before the last menstrual period and continues for 8–10 years. In a recent study it has been shown that approximately 30–40% of cortical bone is lost between 56 and 70 years of age at a uniform rate of 2·5% per year (Quigley *et al.* 1987). As reviewed by Mazess (1982) data on trabecular bone loss are conflicting. In a longitudinal study based on computerized tomography measurements, the rates of trabecular loss at the centrum of the vertebra in the initial stages of menopause averaged 10% per year while longitudinal studies using dual photon absorptiometry (DPA) suggest a loss of about 3–6% annually. By contrast, other studies, cross-sectional and longitudinal, showed a rate of loss of 0·6–1·2% per year throughout (Riggs & Melton 1986*a*).

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Cortical bone remodelling with ageing and the menopause results in net periosteal apposition and endosteal resorption with resultant thinning of the cortex. The medullary cavity increases in size, as does the outer diameter of bone. Trabecular bone is present in substantial amounts in the vertebrae, proximal femur, distal radius and proximal humerus. The 'honeycomb' structure of plates confers a large surface to volume ratio. Trabecular bone has a higher turnover than cortical bone, and is more sensitive to age-related and hormonal factors. Sites with a high ratio of trabecular to cortical bone are more prone to osteoporotic fractures.

Factors increasing the risk of osteoporosis

Genetic and environmental factors contribute to bone mass and there are comprehensive reviews of environmental influences (Cummings *et al.* 1985; Riggs & Melton 1986*a*). A number of factors which are potentially amenable to intervention require emphasis.

Although dietary calcium deficiency may cause osteoporosis in animals, this has never been conclusively proven in man (Riggs *et al.* 1967; Wootton *et al.* 1979). Postmenopausal calcium supplementation has been reported to slow the rate of cortical bone loss (Recker *et al.* 1977; Horsman *et al.* 1977). By contrast, Riis *et al.* (1987) found that daily oral calcium supplementation of 2 g had a minor effect in retarding cortical bone loss compared with placebo and had no effect on retarding trabecular bone loss. Riggs *et al.* (1986*b*) found that the rates of bone loss at the mid-radius and lumbar spine were independent of dietary calcium intake. Thus the widespread use of calcium supplementation does not appear to be warranted.

Daniell (1976) showed that tobacco consumption is a risk factor for osteoporosis. Metabolites of tobacco competitively inhibit aromatase and decrease oestrogen production from weak androgens (Barbieri *et al.* 1986). In addition, inactive hydroxylated metabolites are formed. Women who smoke cigarettes have an earlier menopause (Jick *et al.* 1977) and lower luteal phase oestrogens (MacMahon *et al.* 1982). Postmenopausal women who smoke and are given oestrogens have lower circulating oestrogen levels (Jensen *et al.* 1985). Thus tobacco is a well-established and remediable risk factor for osteoporosis. This has been understated previously.

Alcoholism has also been incriminated as a risk factor for osteoporosis and hip fractures in women (Bikle *et al.* 1985). This may be due to a direct effect of alcohol on bone, associated poor nutrition and thinness, reduced physical activity, predisposition to falls and increased cigarette smoking. Heaney & Recker (1982) reported an association between caffeine intake and negative calcium balance in postmenopausal nuns, but Daniell (1976) found no significant association between heavy coffee consumption and reduced cortical bone mass after adjusting for cigarette smoking and body weight. Several medications may decrease bone density. These include corticosteroids, anticonvulsants, heparin, thyroid hormone and antacids.

Body weight may be an important determinant of bone mass. Patients with lower body weight have more rapid bone loss (Daniell 1976). Obesity protects against osteoporosis in men and women (Seeman *et al.* 1983; Daniell 1976). Obesity also diminishes the increased risk of hip fractures associated with tobacco consumption, and postmenopausal obese women do not appear to derive any protective benefit against hip fractures from oestrogen prophylaxis (Williams *et al.* 1982). This information should be taken into consideration when oestrogen supplements are to be administered. The effects of body weight on bone density are incompletely understood. Conversion of weak androgens to oestrogens in adipose tissue and mechanical factors may be important.

Measurement of bone mass

Major advances in the study of metabolic bone disease have come from the development of non-invasive techniques which allow precise determination of bone mass at the axial and appendicular skeleton.

Single photon absorptiometry (SPA) quantitates bone mineral content of the appendicular skeleton at the mid radius which contains over 90% cortical bone and the distal or ultradistal radius which contain 25 to 75% of trabecular bone (Schlenker & Von Seggen 1976). Radius bone mineral content correlates well with the mineral content of the hip and entire skeleton but poorly with that of the spine (Cummings *et al.* 1985), and a normal value does not exclude spinal osteoporosis (Wahner *et al.* 1984).

Dual photon absorptiometry (DPA) is a technique which measures the total cortical and trabecular bone mineral density at the regions of interest in osteoporosis, the spine and proximal femur. The coefficient of variation of this technique is 2–3% and the radiation exposure is one-tenth that of a standard chest radiograph.

Computerized tomography (CT) selectively measures bone mineral density of the vertebral trabecular bone. CT scanning exposes the patient to a higher radiation dose (over 2000 μSv or 200 mrad) and may underestimate trabecular bone mass unless dual-energy techniques are used (Health and Public Policy Committee, American College of Physicians 1984).

Neutron activation analysis measures total body calcium. This technique is precise and accurate but is available in very few centres.

DPA and CT scanning appear to be the methods of choice for assessment of bone mass. Both provide information regarding bone mass at the spine, and DPA provides information regarding bone mass at the proximal femur. The modest correlations between axial and appendicular sites preclude extrapolation from one site to another.

The most important application of these methods is in the treatment of established osteoporosis, where the efficacy of a given treatment can be quantitated precisely. There is considerable controversy regarding the use of these methods in screening (Ott 1986; Hall *et al.* 1987). At present, selective screening only of persons at risk is recommended. Risk factors for osteoporosis include: (1) increasing age; (2) female; (3) white race; (4) leanness; (5) inactivity; (6) tobacco; (7) alcohol; (8) diseases; (9) family history (?); (10) dietary factors (?)—calcium deficiency, protein excess, phosphorus excess, caffeine, non-fluoridated water; (11) excessive physical activity; (12) corticosteroids, anticonvulsants, thyroid hormone. Population screening should not be undertaken until there is a better understanding of the clinical significance of a low bone mass.

Effect of premenopausal ovulatory disorders on bone mass

As oestrogen deficiency accompanying the menopause is implicated in the development of osteoporosis, it is appropriate to consider whether oestrogen deficiency states in the premenopausal woman, or pregnancy and lactation, which may follow ovulation induction therapy in such women, have adverse effects on bone mass. The suggested mechanisms contributing to the occurrence of osteoporosis in these conditions are shown in Fig. 1.

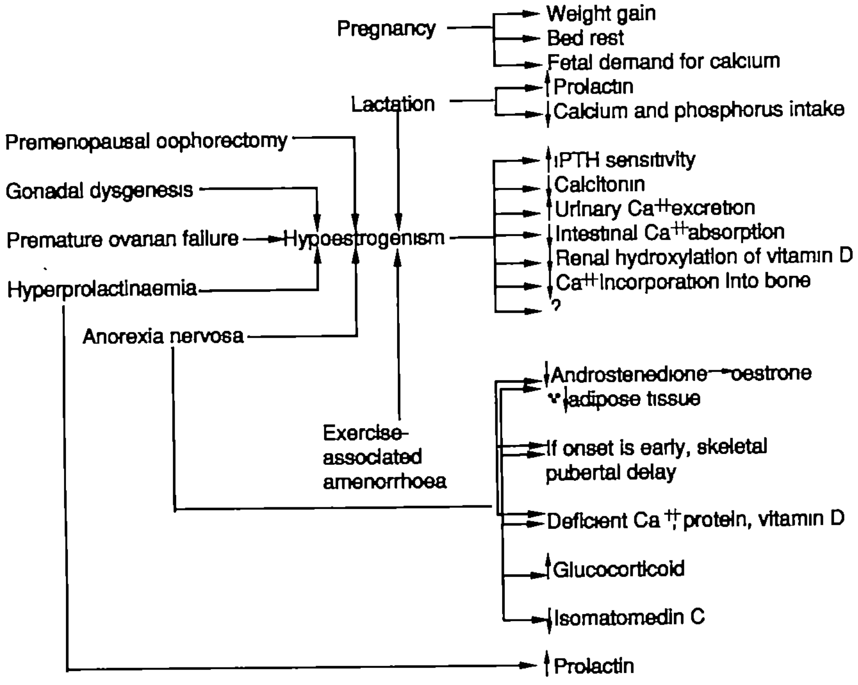
Pregnancy
Weight gain
Bed rest
Fetal demand for calcium
Lactation
↑Prolactin
↓Calcium and phosphorus intake
Premenopausal oophorectomy
Gonadal dysgenesis
Premature ovarian failure
Hypoestrogenism
Hyperprolactinaemia
↑iPTH sensitivity
↓Calcitonin
↑Urinary Ca⁺⁺excretion
↓Intestinal Ca⁺⁺absorption
↓Renal hydroxylation of vitamin D
↓Ca⁺⁺incorporation into bone
?
Anorexia nervosa
Exercise-associated amenorrhoea
↓Androstenedione→oestrone via ↓adipose tissue
If onset is early, skeletal pubertal delay
Deficient Ca⁺⁺, protein, vitamin D
↑Glucocorticoid
↓Isomatomedin C
↑Prolactin

Fig. 1. Possible mechanisms in the development of osteoporosis in gynaecological disorders.

Premenopausal oophorectomy and the effect of oestrogen replacement

Although Davis *et al.* (1966), Meema & Meema (1968) and Nordin *et al.* (1968) suggested that oestrogen therapy given after oophorectomy may delay the onset of bone loss, it was not until the 1970s that this was shown to be correct. Aitken *et al.* (1973*a*) found a significant increase in bone loss in oophorectomized patients only when surgery was done before the age of 45 years. Oestrogen therapy effectively prevented bone loss if started within 2 months of oophorectomy. If started 3–6 years after surgery, the bone loss may be replaced. Beyond 6 years the bone loss was not replaced and further bone loss was not prevented (Aitken *et al.* 1973*b*). The earlier the oestrogen is initiated after the oophorectomy, the more pronounced are the beneficial effects on bone loss (Abdalla *et al.* 1984). These effects lasted as long as oestrogen was given. On cessation of therapy rapid bone loss resumed (Lindsay *et al.* 1978).

In a comparative trial of oestrogen- and placebo-treated patients, assessed after a median follow-up of 9 years, the placebo-treated group showed a significant reduction in spinal wedge angles, peripheral bone mineral content, and a reduction in stature whereas these changes were not observed in the oestrogen-treated group. However, the protective effect of oestrogen on bone loss lasted for up to only 8–10 years of continuous administration after which there was evidence of a small but significant bone loss (Lindsay *et al.* 1980).

Work by Christiansen *et al.* (1981) on the natural menopause also supports the efficacy of oestrogen administration in reducing osteoporosis after loss of ovarian function. Other studies using CT scanning of the spine showed significant vertebral bone loss 6 months after oophorectomy and as much as 12% in the first 2 years (Cann *et al.* 1980; Genant *et al.* 1982).

Gonadal dysgenesis (Turner's syndrome)

Osteoporosis has been described in patients with gonadal dysgenesis and has been attributed to oestrogen deficiency (Albright *et al.* 1942; Levin 1962; Greenblatt *et al.* 1967). However, hypoestrogenism is probably not the sole explanation as osteoporosis has also been reported in children before puberty with gonadal dysgenesis (Lemlie & Smith 1963; Greenblatt *et al.* 1967; Goldberg *et al.* 1968). The normal pubertal exponential increase in bone mineral density fails to occur in gonadal dysgenesis despite a normal adrenarche. Women with gonadal dysgenesis have significantly less bone mineral density at age 20 than age-matched controls, but increases in bone mineral density occur with oestrogen therapy (Garn 1970).

Premature ovarian failure

Cann *et al.* (1984) demonstrated a significant decrease of 20·9% in spinal trabecular bone density measured by CT scanning in 16 women with premature ovarian failure compared with normally menstruating control women. There was also a slight decrease in peripheral cortical bone density measured by SPA. The bone loss was not related to the duration of amenorrhoea or serum oestradiol concentrations. However, Jones *et al.* (1985) found an inverse correlation between peripheral bone density and the duration of amenorrhoea in women with premature menopause.

Hyperprolactinaemia

Klibanski *et al.* (1980) reported a significant reduction in bone density measured by SPA in 14 hyperprolactinaemic women which correlated inversely with serum oestradiol but not with serum prolactin. Moreover, the bone densities of patients with the lowest oestrogen values were lower than those of postmenopausal women. Whereas significant bone loss in hyperprolactinaemia has been confirmed by other workers using SPA and CT, especially for spinal trabecular bone, the inverse correlation between bone density and oestrogen level has not been confirmed (Shlechte *et al.* 1983; Cann *et al.* 1984). In a study of 60 women, 53 of whom had had bromocriptine therapy, significant bone loss was demonstrated by DPA in the lumbar spine but not in the femoral neck or shaft. The bone loss was associated with the presence, but not the duration, of amenorrhoea and parity appeared to offer some protection (Pepperell & Seeman 1985). Klibanski & Greenspan (1986) studied the radial bone density by SPA in 32 hyperprolactinaemic women, 18 after treatment with surgery or bromocriptine resulting in restoration of menses and ovulation. After therapy, bone density increased significantly but remained lower than control values, while the bone density of the untreated patients decreased significantly over time. Similar results were obtained in 18 hyperprolactinaemic men by the same group. Six of the seven men with longitudinal follow-up showed a significant increase in forearm bone density after normalization of serum prolactin or testosterone levels (Greenspan *et al.* 1986).

The pathogenesis of bone loss associated with hyperprolactinaemia is incompletely understood. The positive correlation between bone density and serum oestradiol concentrations led Klibanski *et al.* (1980) to conclude that the reduced bone density was probably due to relative or absolute oestrogen deficiency. However, hypoestrogenaemia is not a uniform finding in hyperprolactinaemia and the failure of other workers to confirm the correlation between bone density and oestrogen levels suggests that other factors may be involved (Schlechte *et al.* 1983).

Anorexia nervosa

Ayers *et al.* (1984) found retardation of bone age by at least 2 years and significant reduction in cortical thickness using radiogrammetry in 14 patients with secondary amenorrhoea and anorexia nervosa. The bone loss was related to the duration and age of onset of amenorrhoea. The authors suggested that the reduction in cortical thickness may be due to failure to undergo the normal pubertal apposition of cortical bone leading to prolonged ‘skeletal pubertal delay’ rather than active bone resorption. Rigotti *et al.* (1984) found that radial bone mineral density was significantly reduced in 18 women with anorexia nervosa. The changes were not related to oestradiol levels. Two girls aged 18 and 24 had vertebral compression fractures. Five girls with high physical activity (defined as vigorous exercise at least three times weekly) had bone density which was comparable to controls and significantly greater than that of the less active girls. The authors concluded that physical activity may protect against bone loss in patients with anorexia nervosa. Bone loss associated with anorexia nervosa has also been reported in other studies and is particularly marked in patients with anorexia nervosa of more than 10 years duration (Cann *et al.* 1984; Jones *et al.* 1985; Szmukler *et al.* 1985). A recent study showed that the significant bone loss associated with anorexia nervosa was related to the duration of the condition, improved with weight gain and completely returned to normal with recovery (Treasure *et al.* 1987).

The pathogenesis of bone loss in anorexia nervosa is probably multifactorial. Factors which have been implicated include deficiency of oestrogen of ovarian origin or from reduced peripheral conversion of adrenal androstenedione to oestrone in adipose tissue, dietary deficiency of protein, calcium or vitamin D (Fonseca *et al.* 1985), excessive glucocorticoid secretion (Gallagher & Riggs 1978; Gerner & Gwirtsman 1981), hyperprolactinaemia, decreased levels of insulin-like growth factor 1 (somatomedin C) with decreased bone collagen formation as in starvation (Clemmons *et al.* 1981), and the failure to achieve normal pubertal apposition of cortical bone (Ayers *et al.* 1984) and optimal skeletal consolidation (Aitken 1985) in patients with early onset of this disease.

Exercise-associated amenorrhoea

Decreased bone mass in postmenopausal women may be reversed by regular exercise (Aloia *et al.* 1978; Smith *et al.* 1981; Krolner *et al.* 1983). A study using CT scanning of first lumbar vertebra in long-distance runners over 50 years of age showed 40% higher bone mineral in both sexes than matched controls (Lane *et al.* 1986). By contrast, vigorous exercise may have a deleterious effect on bone mass if associated with amenorrhoea. Cann *et al.* (1984) found that the spinal trabecular bone in 10 subjects with amenorrhoea who exercised frequently was decreased by more than 20% compared with controls. Bone mass in the peripheral skeleton in this group was only slightly decreased. Similar disparate changes at axial and appendicular sites in amenorrhoeic athletes have been reported by others (Drinkwater *et al.* 1984; Jones *et al.* 1985). The rate of spinal bone loss in the first 3 years of exercise-induced amenorrhoea was also found to be greater than that found in subjects with chronic exercise-associated amenorrhoea. Based on this data, Cann *et al.* (1985) suggested that treatment of amenorrhoeic athletes with oestrogens to prevent bone loss, like treatment after oophorectomy, should be initiated early to be effective.

Drinkwater *et al.* (1984) found lower mean and peak oestradiol, prolactin and peak progesterone levels in amenorrhoeic athletes. The hormonal levels did not correlate with bone mass. In addition, excessive thinness and amenorrhoea may predispose female athletes to reduced bone mass (Linnell *et al.* 1984).

Pregnancy and lactation

The average calcium content of the full-term baby is 20 g and the calcium lost during pregnancy is 30 g (Albright & Reifenstein 1948; Fourman & Royer 1968). This quantity should not cause any appreciable disturbance to the maternal skeleton calcium store of about 1000 g. Calcium demands during lactation are greater as 500 ml of milk contains 400 mg of calcium. If the mother were to lactate for 9 months, four times as much calcium would be lost in the breast milk as is lost during pregnancy (Albright & Reifenstein 1948). Normally complex regulatory mechanisms act to protect the pregnant woman's bones from excessive resorption. During pregnancy, intestinal absorption and renal reabsorption of calcium are increased (Shenolikar 1970; Gross 1962). The effects of normal pregnancy and lactation on serum total and ionic calcium, parathyroid hormone, calcitonin, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and on bone mass are contradictory (Retallack *et al.* 1977; Pitkin *et al.* 1979; Stevenson *et al.* 1979; Kovarik *et al.* 1980; Palmieri 1981; Dent & Gupta 1975; Meunier & Bressot 1982; Weiseman *et al.* 1978; Kumar *et al.* 1979). Three retrospective studies showed an increase in bone mass with parity (Smith 1967; Nilsson 1969; Goldsmith & Johnston 1975). One prospective study revealed constant bone mineral content during pregnancy (Christiansen *et al.* 1976) while another showed significant bone loss (Lamke *et al.* 1977). Prospective studies are required to resolve these controversies.

A syndrome of post-pregnancy osteoporosis was first suggested by Nordin & Roper (1955). They reported four patients with spinal osteoporosis of varying degree. Dent & Friedman (1965) reported five women with severe vertebral fractures after delivery. They argued that pregnancy was most likely coincidental, the fractures occurring with pre-existing osteoporosis because of the weight gain during pregnancy. A study using quantitative bone histomorphometry in three patients with spinal osteoporosis associated with pregnancy and lactation failed to demonstrate the typical high turnover osteoporosis exhibited by some juvenile or postmenopausal osteoporotics (Gruber *et al.* 1984). Beaulieu *et al.* (1976) reported transient osteoporosis of the hip beginning shortly before or during the third trimester of pregnancy and associated with elevated urinary hydroxyproline. Whether these findings are causally or incidentally associated with pregnancy is unknown. In the few follow-up studies conducted, there was no disease progression associated with subsequent pregnancies. We have described one young woman with severe spinal osteoporosis and multiple factors which may have contributed to the bone loss (Lam *et al.* 1987).

Effect of oral contraceptives

There are few reports on the effects of oral contraceptives on bone. In one study young women in their twenties using oral contraceptives containing high doses of mestranol were found to have enhanced bone mineralization and the women who lactated benefited most from the therapy (Goldsmith & Johnston 1975). A later study also found greater cortical bone mass with the use of oral contraceptives (Kanders *et al.* 1984).

Comment

As no effective treatment is available for replacing bone already lost, preventive care assumes an important place in health care planning. This includes the recognition, counselling and treatment of those at risk for osteoporosis before bone loss and fracture occur. During the last decade, it has become possible to measure bone density in the vertebrae and hip using non-invasive and precise techniques.

All the hypogonadal and ovulatory disorders reviewed are associated with a hypoestrogenic state and some clearly predispose to bone loss. Premenopausal oophorectomy, premature ovarian failure, gonadal dysgenesis, hyperprolactinaemia, anorexia nervosa and exercise-induced amenorrhoea are well-documented causes of bone loss while the effect of other ovulatory disorders, pregnancy and lactation is still unclear. The number of women included in these groups is substantial. For example, in Australia and New Zealand, disorders of ovulation are observed in 20% of infertile women (Pepperell 1986). In the Royal Women's Hospital 10% of the women with secondary amenorrhoea attending the endocrine clinic are suffering from premature ovarian failure or ovarian resistance, 20% from hyperprolactinaemia, 10% polycystic ovary syndrome, and 60% from hypothalamic amenorrhoea that may have been precipitated by weight change, stress, illness or hormone administration but is generally of unknown cause.

We have emphasized the importance of risk factors including some gynaecological disorders which act at least partly through oestrogen deficiency. There are important clinical implications. Women with anovulation usually require ovulation induction to conceive and are therefore subjected to a higher risk of multiple pregnancies, yet the effect of pregnancy, lactation and oral contraceptives on bone mass are not well established. If pregnancy and lactation are risk factors for bone loss, multiple pregnancy in these hypoestrogenic women will compound the risk. Therefore it is important to clarify the effect of pregnancy and breast-feeding on bone mass in prospective studies.

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The management of the patient with abnormal vaginal cytology following hysterectomy

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Summary. Thirty-two patients presenting with abnormal vaginal cytology following hysterectomy were studied. Seven (21·8%) had had hysterectomy for benign conditions whilst 25 (78·1%) had cervical intraepithelial neoplasia (CIN) or invasive cervical carcinoma. Twenty-five patients had partial or total vaginectomy (15 as the primary procedure), and one required laser treatment following vaginectomy. Of 11 (34·3%) patients treated primarily by laser, five subsequently required vaginectomy because of persistent or recurrent cytological abnormality. All four patients treated with topical 5-fluorouracil or dinitrochlorobenzene subsequently required surgery. Nine of the 32 patients (28·1%) proved to have invasive carcinoma of the vagina on histological examination of the vaginectomy specimen. At the time of writing all patients in the study are well with no evidence of disease.

Vaginal intraepithelial neoplasia (VAIN) is said to occur following hysterectomy for cervical intraepithelial neoplasia (CIN) in between 1 and 6% of cases (Parker *et al.* 1960; Petrilli *et al.* 1980; Woodman *et al.* 1984). It is less commonly observed following hysterectomy for benign disease. With the advent of colposcopy and the gynaecological laser, there has been a move away from the traditional approach of partial or total vaginectomy for these lesions. Unfortunately, in published series on laser treatment the numbers are small, the follow-up period is short and the cure rate is disappointing (Stafl *et al.* 1977; Petrilli *et al.* 1980; Townsend *et al.* 1982; Woodman *et al.* 1984).

Patients and methods

The case records of 32 patients referred to the Regional Department of Gynaecological Oncology, Gateshead, over a 14-year period, 1974–1987, were reviewed. All these patients had had a hysterectomy previously and presented with abnormal vaginal cytology. Most of them were assessed colposcopically and in all of them Schiller's iodine was used to define iodine-negative areas in the vagina.

A variety of different first-line treatments was employed. These included partial or total vaginectomy, local excision, laser therapy, topical 5-fluorouracil (5 FU) treatment, and 2-4-dinitrochlorobenzene (DNCB) application. The technique used for partial and total vaginectomy was that described by Monaghan (1986).

Results

The mean age of the patients at the time of presentation was 50·1 years (range 33–70 years). Seven of the 32 (21·8%) had had their hysterectomies for benign conditions (fibroids, menorrhagia); 21 (65·6%) had evidence of CIN on the hysterectomy specimen, whilst 4 (12·5%) had invasive carcinoma. Of those with no history of CIN, the average time to development of the abnormal vaginal smear was 11·3 years (range 4–21 years). Of the 21 women who had CIN, 8 (38·1%) demonstrated an abnormality on the first vaginal smear taken after the hysterectomy. The longest interval from hysterectomy to abnor-

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mal vaginal smear in this group was 10 years. Of the four patients with invasive carcinoma, one had an abnormal vaginal smear at the first follow-up, another after 1 year, but the other two went 12 and 15 years respectively before developing abnormal vaginal cytology.

Of the 32 patients, 15 (46·8%) had partial or total vaginectomy as a primary procedure whilst a further 10 had vaginectomy following failure of other primary treatment. Eleven patients had vaginectomy on the basis of persistently abnormal cytology, without histological confirmation of an abnormality, but only three of them had a negative histology report on the vaginectomy specimen. Those who had total vaginectomy had a split thickness skin graft at the time of the procedure and one had this carried out as an interval procedure. In one case vaginectomy proved to be technically not possible, this patient eventually requiring exenteration. Only one patient required further treatment following vaginectomy; the lesion proved to be VAIN 1 and was successfully treated with the laser.

Partial or total vaginectomy is never an easy procedure and may on occasions be very difficult due to scarring from previous surgery, but the morbidity in this study was very low. No patients had a ureteric or vesico-vaginal fistula. The main problem encountered was bladder hypotonia; five patients required prolonged catheterization, but at the time of writing, all but one can now void successfully. Most were able, eventually, to resume normal sexual activities, although this aspect was not fully recorded in every case.

Of the 11 patients (34·3%) treated primarily with the laser, five required further treatment subsequently because of persistent or recurrent vaginal smear abnormality. Three of the five had abnormal cytology on the first smear after laser treatment. Two patients were treated with 2-4-dinitrochlorobenzene and two with 5-fluorouracil, all four of them requiring surgical intervention subsequently. Nine of the 32 (28·1%) proved to have invasive carcinoma of the vagina on histological examination of the vaginectomy specimen. Four of these were microinvasive lesions. In five women epithelium was lost after vaginectomy, this was thought to be due to the packing technique which lifted off the epithelium.

For the 15 patients who had vaginectomy as a primary procedure, the median length of follow-up was 2 years (range 6 months –10 years), whilst for the 10 who had vaginectomy following other primary treatment, the median follow-up was 1 year (range 3 months–11 years). Three of these 25 patients have not yet had a follow-up smear, but the other 22 have had at least one negative vaginal smear following treatment. Of the six patients treated by laser alone, half have not yet had subsequent cytology, the maximum period of follow-up being 1 year. Of the total group 19 (59%) were first seen with abnormal vaginal cytology within the preceding 5 years.

Discussion

Although uncommon, dysplastic abnormalities of the vaginal epithelium after hysterectomy demand careful assessment and management. There are three possible explanations for the pathogenesis of the condition (Woodruff 1965): (i) Residual disease indicating inadequate excision of the original lesion. (ii) Multicentre focus of origin. (iii) New or recurrent lesion due to a persistent carcinogenic stimulus or possibly resulting from therapy for the initial lesion (e.g. radiation).

For the group with no previous history of CIN, the third explanation is the most likely, particularly as the interval after hysterectomy was long in all cases. This indicates the need for continuing cytological follow-up, even after hysterectomy for benign conditions. It would appear reasonable to take these smears at 3-year intervals. For the CIN group, however, the failure to achieve a normal vaginal smear after hysterectomy in a substantial number of patients suggests that the original lesion was inadequately assessed and incompletely excised.

Several authors have produced data which support a multicentric origin of dysplastic disease of the cervix, vagina and vulva (Ferguson & Maclure 1963; Woodruff 1965; Hummer *et al.* 1970; Hammond & Monaghan 1983). Wade-Evans (1976) stated 'a histologically and cytologically normal epithelium at the edge of a demonstrably dysplastic area already carries within it seeds of further malignancy . . . the fault may lie in abnormality of the suppporting mesenchyme rather than the epithelium itself'. If the multicentric origin theory is accepted, albeit as a rare occurrence, it is clearly essential to assess the cervix and the vaginal fornices very carefully at any colposcopic examination for an abnormal cervical smear. This is particularly the case if no lesion is found on the cervix itself or if it is planned to carry out a simple hysterectomy for CIN. With the technique normally employed for simple hysterectomy no significant amount of vaginal epithelium is removed and therefore if the cervical lesion extends onto the vagina, it will be incompletely removed by this technique. The addition of a so-called 'cuff' often succeeds only in removing a small portion of vagina at the front and back and none at the sides. A suitable alternative would be to combine simple hysterectomy with laser ablation of the peripheral part of the lesion or to carry out a modified radical hysterectomy where the ureters are dissected and displaced, allowing a proper cuff of vagina to be removed. If a degree of prolapse is present, vaginal hysterectomy may be appropriate and allows accurate removal of affected vaginal skin.

When assessing the vagina after previous hysterectomy, it is necessary to apply iodine to the whole vagina in order to identify any iodine-negative areas which may be indicative of dysplastic change. Biopsies should be carefully taken of these areas and their position meticulously documented. The vault of the vagina is especially difficult to assess. Abnormal epithelium may be sequestered above the suture line and the 'angles' often contain deep pockets of epithelium which cannot be fully assessed. Bimanual palpation is vitally important after colposcopy as occasionally a tumour may be palpable above the vault suture line. The authors advocate partial vaginectomy wherever abnormal epithelium is seen at the angles of the vault. As this study shows, in a substantial proportion of patients, *invasive* carcinoma had developed, and may well have gone totally undetected until well advanced, if a surgical approach had not been used. The amount of vaginal tissue to be removed should be assessed carefully and only as much tissue as is necessary should be removed. This may mean only 1–2 cm at the top of the vagina which will not jeopardise future sexual function. Early invasive lesions may be adequately excised with the radical technique described by Monaghan (1986). For those few patients where total vaginectomy is felt necessary, skin grafting to create a new vagina should be seriously considered.

Laser treatment was clearly ineffective in almost half the patients in this series; all of the laser 'failures' had lesions at the vault. We feel that there is a place for laser treatment, but only for lesions which do not involve the 'angles' and where biopsies, taken with care, have demonstrated no evidence of invasion. Radiotherapy to treat VAIN has been advocated by some authors, as being preferable to the technically difficult operation of partial or total vaginectomy. However, the morbidity from radiotherapy is clinically significant, particularly vaginal stenosis, bowel and bladder effects and in some patients, loss of ovarian function.

We feel strongly that partial or total vaginectomy should be performed in a gynaecological oncology centre by those skilled in radical pelvic surgery. In that case, this study has shown that morbidity is relatively minor and the success rate is high. In selected patients laser treatment of localized areas of abnormality in the vagina may be combined with the partial vaginectomy.

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Radiotherapy in the management of vaginal intraepithelial neoplasia after hysterectomy

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Summary. Intravaginal radium was used to treat 11 patients who were found to have vaginal intraepithelial neoplasia (VAIN) following hysterectomy for cervical intraepithelial neoplasia. All were discovered by cytological follow-up, were colposcopically assessed and diagnosis was confirmed by histological examination before treatment. Nine of 10 patients questioned continue to enjoy satisfactory sexual intercourse after treatment but 6 of 8 premenopausal patients subsequently became oestrogen deficient and required hormone replacement therapy. No other significant morbidity has been observed. All remain cytologically and colposcopically free of disease after a median follow-up of 26 months. We conclude that radiotherapy is an effective treatment of VAIN following hysterectomy.

The frequency of vaginal intraepithelial neoplasia (VAIN) after hysterectomy for cervical intraepithelial neoplasia (CIN) has been reported to be between 0·8 and 5·6% (Wade Evans 1976; Lee & Symmonds 1976; Funnell & Merrill 1963). The management of these lesions remains problematic. Some authors have suggested partial or total vaginectomy with split thickness skin grafting as the treatment of choice (Gallup & Morley 1975; Schiffer *et al.* 1972), but these difficult procedures are not always effective (Gallup & Morley 1975; Oliver 1979). We have reported disappointing results with the use of the CO_2 laser in the treatment of 14 patients with VAIN after hysterectomy (Woodman *et al.* 1984). An extended period of follow-up has revealed that only five patients remain free of disease, six required alternative forms of treatment to eradicate residual intraepithelial neoplasia and a further three were subsequently found to have invasive carcinoma of the vaginal vault. The aim of this study was to assess prospectively the effectiveness of radiotherapy in the treatment of VAIN after hysterectomy.

Patients and methods

All patients were referred to the colposcopy clinic at the Birmingham and Midland Hospital for Women when cytological examination of the vaginal epithelium showed abnormality to persist after hysterectomy for carcinoma-in-situ of the cervix (CIN III). The initial assessment was conducted in the outpatient department in every case. When this suggested the presence of epithelial abnormality the patient was admitted to hospital and under general anaesthesia a colposcopically directed biopsy was taken from the vaginal vault. If histological examination of this material revealed VAIN, the patient was referred to the department of radiotherapy in the Queen Elizabeth Hospital for the intravaginal insertion of radium. All patients were examined under general anaesthesia and the bladder was catheterized before insertion of radium. The

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technique of radium insertion was determined by the anatomical configuration of the vaginal vault and the site of VAIN. In 10 patients the disease was confined to the vaginal vault, and one or two cylinders (measuring 3 cm in length and 2 cm in diameter), each containing either 15 or 20 mg of radium, were inserted into the vaginal vault, which was then packed with a gauze roll soaked in proflavine cream. In the remaining patient the disease extended to the middle third of the anterior vaginal wall and a cylinder (measuring 8 cm in length and 2·5 cm in diameter), containing 30 mg of radium, was used to irradiate the whole length of the vagina; this was secured in place by a vulval suture. The duration of insertion was 72, 96 or 120 h, depending on the amount of radium used and patient tolerance of the procedure. Calculations of the dose of absorbed radiation were made retrospectively. After the treatment was completed the radium source was removed and the patient was discharged the same day. All patients were instructed in the use of vaginal dilators and encouraged to resume sexual intercourse after an interval of 4 weeks.

Patients were reviewed in both the radiotherapy and colposcopy clinics 6 weeks after treatment. Subsequent review was by clinical, cytological and colposcopic assessment at intervals of 6 months.

Results

The median age of the 11 patients in this series was 39 years (range 34–65 years).

Site of lesion

Colposcopic examination showed the lesion to involve one or both vaginal angles in nine patients and in one of them the lesion extended to involve the upper third of the anterior vaginal wall. In the remaining two patients the lesion straddled the vault suture line but did not involve the vaginal angles.

Histology

Histological examination of the colposcopically directed biopsy revealed squamous carcinoma-in-situ in 10 patients (VAIN III). The remaining patient was found to have adenocarcinoma-in-situ although review of the sections from the cone biopsy and hysterectomy specimens revealed only squamous intraepithelial neoplasia.

Dose of absorbed radiation

The mean dose of absorbed radiation at a distance of 1 cm from the surface of the radium cylinders was 3611 cGy (range 2448–4080).

Morbidity

Early. At first review three patients complained of profuse vaginal discharge which resolved after treatment with Hydrargaphen pessaries; three complained of diarrhoea which occurred within 2 weeks of treatment and had resolved by the time of first review in two but persisted for 8 months in the remaining patient. One patient complained of mild cystitis following treatment but this had resolved before the first review.

Late. Clinical and colposcopic examination has revealed no patient with vault stenosis. Ten patients were asked about possible difficulties with sexual intercourse after treatment; nine continue to enjoy satisfactory sexual intercourse; in the remaining woman all of the vagina had been treated because of extensive disease, and the patient complains of persistent deep dyspareunia. Of the eight premenopausal patients six required hormone replacement therapy because of oestrogen deficiency which was suggested by climacteric symptoms and confirmed by elevated follicle-stimulating hormone levels.

Outcome

Regular clinical, cytological and colposcopic review has shown that all patients remain free of disease after a median follow-up of 26 months (range 16–36 months).

Discussion

VAIN after hysterectomy for CIN most frequently follows the incomplete excision of an abnormal cervical transformation zone which extends onto the vagina. The usual method of closing the vaginal vault after hysterectomy secures haemostasis by apposing the vaginal edges, which inevitably results in some vaginal epithelium being buried above the suture line. We believe the use of CO_2 laser, coagulation electrodiathermy or local excision is inappropriate in the management of VAIN after hysterectomy as atypical epithelium may be inaccessible because it is buried in the recesses of the vaginal angles or sequestered about the vault suture line (Woodman *et al.* 1984). The 11 patients in this series remain cytologically and colposcopically free of disease after a median follow-up of 25 months. The dose of absorbed radiation at a distance of 1 cm above the epithelial surface of the vault ranged from 2784 to 4080 cGy. This was considerably less than that used by Hernandez-Linares *et al.* (1980) who reported no recurrences in 28 patients when the radiation dosage ranged from 8160 to 16 320 cGy at a distance of 1 cm above the vault but more than that used by Punnonen *et al.* (1981) who found no evidence of residual disease in nine patients when the radiation dosage ranged from 2000 to 2600 cGy at a distance of 1 cm from the surface of the epithelium. Comparison with other studies is frustrated because no distinction is made between the outcome in patients in whom VAIN was preceded by invasive malignancy and the outcome in those who developed VAIN after hysterectomy for pre-invasive disease (Brown *et al.* 1971).

There has been a reluctance to use radiotherapy in the management of the younger patient because of the risk of impairing sexual function. This may explain why the mean age of patients in this series is almost 10 years younger than that reported elsewhere (Punnonen *et al.* 1981; Brown *et al.* 1971).

Undoubtedly the irradiation or removal of more than half the vagina by partial vaginectomy will produce shrinkage and loss of functional capacity, but there is little evidence to suggest that this is an inevitable sequel to the use of intravaginal radium in the treatment of localized vault lesions. Punnonen *et al.* (1981) reported no immediate or late complications following the use of radium in nine patients with VAIN but did not make specific reference to sexual function. Oliver (1979) reported vaginal shortening and stenosis in two of four patients treated with an unspecified dose of intravaginal radiotherapy but they claimed that the subsequent use of vaginal dilators and the topical application of oestrogen creams restored sexual function in the women. In the present series vaginal stenosis has been almost completely prevented by the use of vaginal dilators and early resumption of sexual activity. This has not only permitted adequate coitus in nine patients but has also facilitated cytological and colposcopic surveillance of the vault mucosa. We have no evidence of delayed vault stenosis and believe that this can be avoided if the acute reaction is managed correctly. Late reactions involve the submucosal tissues and may produce some shrinkage of the vagina but not the formation of adhesions. While loss of ovarian function would appear almost inevitable, this can be offset by hormone replacement therapy. The empirical dose levels used in this study appeared adequate to eradicate surface disease but it may be premature to consider these patients cured as atypical epithelium above the suture line may not have been adequately destroyed. In addition, the theoretical risk of radiation-induced pelvic malignancy must be considered. The potential sites of induced malignancy are those receiving relatively low doses of radiation. It is therefore unlikely that vaginal malignancy will be induced as this receives a high dose of radiation. We cannot rule out the possibility of pelvic malignancy developing in low-dose areas such as the large bowel, bladder and ovaries. Life-long follow-up will be essential to assess the potential long-term hazard but the lack of morbidity following this technique is sufficiently encouraging to suggest that radiotherapy may be the optimum treatment for VAIN after hysterectomy for pre-invasive disease of the cervix.

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Antenatal fetal heart rate variation in relation to the respiratory and metabolic status of the compromised human fetus

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Summary. Three groups of women were delivered by caesarean section before labour: for an abnormal fetal heart rate (FHR) trace (21 cases, group 1), or for maternal deterioration in severe pre-eclampsia without gross fetal heart rate abnormalities (20 cases, group 2), or to avoid mechanical difficulties in labour at term (30 cases, group 3). The mean gestational ages of the first two groups were 32 weeks with a high proportion of infants small-for-gestational-age. In group 1, FHR variation (mean range of pulse intervals) was less than half (20·6 SE 1·2 ms) of the normal value at the same age (44·4 SE 1·5 ms). This was associated with hypoxaemia (mean umbilical artery Po_2 of 6 mmHg at delivery), with evidence of compensation shown by an elevated amniotic fluid erythropoietin. The fetuses were hypoglycaemic and had greater umbilical artery blood alanine concentrations, but no large changes in adenine nucleotide or endorphin plasma concentrations. Although there was a minor degree of respiratory acidaemia at birth, there was not significant metabolic acidaemia. The results demonstrate that the reduced variation of 'suboptimal' and 'decelerative' fetal heart rate records is associated with fetal hypoxaemia and evidence of nutritional deprivation, but not with asphyxia.

It is difficult to make direct measurements of the biochemical status of the human fetus as its heart rate pattern deteriorates; indirect measurements, using umbilical cord blood taken at caesarean section before the onset of labour, suggest that 'suboptimal' and 'decelerative' patterns (Visser & Huisjes 1977) are not associated with metabolic acidaemia (Henson *et al.* 1983), whereas the terminal pattern is (Visser *et al.* 1980). In 'suboptimal' and 'decelerative'

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records, fetal heart rate (FHR) variation is reduced in all phases of the fetal rest–activity cycle, which appear to be unchanged without prolongation of episodes of low FHR variation (Henson *et al.* 1984). Henson *et al.* (1983) showed that reduced FHR variation was not associated with metabolic acidaemia. In this study we extend these observations to include measurements of Po_2, metabolic and endocrine factors.

We compared three groups of patients delivered by caesarean section: (i) for fetal compromise between 28 and 36 weeks gestation, (ii) for urgent maternal reasons between 28 and 36 weeks, and (iii) electively at 38–40 weeks.

Patients and methods

Three groups of singleton pregnancies, delivered by caesarean section before labour, were studied. The 21 women in the first group (10 nulliparous, 11 multiparous) were delivered preterm at between 28 and 36 weeks for fetal distress as judged by visual assessment of conventional FHR records (20 'decelerative' and one 'suboptimal' as defined by Visser & Huisjes (1977)) and, in some cases, by an ultrasound biophysical profile (Manning *et al.* 1981). In 15 pregnancies there was severe proteinuric pre-eclampsia; in the remainder no cause was found for the intrauterine growth retardation. Eighteen operations were performed under general anaesthesia, three under epidural analgesia.

The 20 women in the second group (13 nulliparous, 7 multiparous) were delivered preterm (28–36 weeks) either for maternal reasons (severe proteinuric pre-eclampsia with symptoms, deteriorating renal function or liver involvement) or to pre-empt complications (for example, of pre-eclampsia) at a gestational age where the risks of preterm delivery were low. In no patient was the FHR record the event that provoked delivery. Eight records were 'suboptimal' and 12 were 'normal'. Seventeen operations were performed under general anaesthesia, three under epidural analgesia.

The 30 women in the third group (3 nulliparous, 27 multiparous) were delivered at 38–40 weeks by caesarean section because of cephalo-pelvic disproportion (11), previous caesarean section (13) or breech presentation (6). All FHR records were normal and there was no other evidence of placental dysfunction. Seventeen operations were performed under general anaesthesia, 13 under epidural analgesia.

Group 2 was chosen to control for gestational age, birth-weight, maternal disease and maternal medication. Thus six women in group 1 and seven in group 2 were treated with methyldopa alone, six women in group 1 and nine in group 2 were given methyldopa and nifedipine, and one woman in each group received methyldopa, nifedipine and labetalol.

The general anaesthetic technique was standardized: pre-oxygenation, left lateral tilt, thiopentone and scoline induction followed by ventilation with nitrous oxide and oxygen after a non-depolarizing muscle relaxant. Hartmann's solution was given intravenously; dextrose solutions were not used. An analgesic was given only after the baby was born. Epidural analgesia was given with bupivacaine, after intravenous loading with Hartmann's solution.

Analysis of fetal heart rate

A FHR record 60 min long was made within 24 h of delivery, using a Hewlett-Packard 8040A monitor, with a Doppler ultrasound transducer (15245A), an abdominal tocodynamometer (15248A) and an event marker (15249A) to identify fetal movements. The data were analysed on-line as described previously (Dawes *et al.* 1985) using a Nascom 2 microprocessor.

FHR variation was calculated as the average over 1 h of the minute-by-minute ranges of the pulse intervals about a computed baseline with decelerations excluded (Dawes *et al.* 1982). Episodes of high and low variation were defined when 5 of 6 successive min had a range of pulse intervals >32 and <30 ms respectively. If, after 25 min no episode of high variation had been identified, the thresholds were reset to 24 and 22 ms to seek rest/activity cycling at a lower level (Henson *et al.* 1984). The basal heart rate was calculated as the mean FHR in episodes of low variation or, if no such episodes were present, as the mean pulse interval expressed as beats/min. The baseline variation was calculated as the maximum pulse interval deviation of the baseline in ms. Fetal movements were recorded when the subject pressed the event marker. No more than one movement every 1/16 min was identified.

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Sample collection and analysis

At operation a clean 5-ml sample of amniotic fluid was collected for measurement of erythropoietin and adenine nucleotide metabolites, transferred to a tube containing EDTA and centrifuged within 60 min. The supernatant was stored at −20°C. Erythropoietin was measured using modification 1 (Cotes *et al.* 1983) of the radioimmunoassay described by Cotes (1982). The samples were first dialysed against 0·02 M veronal buffer at pH 8·6 containing 1:10 000 (w/v) sodium azide. Hypoxanthine, xanthine, uridine and urate concentrations were measured by high-pressure liquid chromatography after trichloracetic extraction (Simmonds & Harkness 1981). Inosine was not detected in any sample, showing that specimens had been obtained without haemolysis.

At delivery the umbilical cord was double clamped and samples of blood were collected for analysis of pH and blood gases (ABL 3, Radiometer). Glucose, lactate and alanine were measured by specific enzymatic methods (Bergmeyer 1974). Intra- and inter-assay coefficients of variation were less than 2·4% and 3·7% respectively, for all three metabolites.

Umbilical blood samples for β-endorphin-like immunoreactivity were collected into ice-cold lithium-heparin tubes and centrifuged for 15 min at 4°C; the plasma was stored at −20°C until assay (Jeffcoate *et al.* 1978*a*,*b*). The assay shows equimolar cross-reactivity with beta lipotrophic hormone.

Complete measurements were not always possible because it was difficult to obtain adequate volumes of blood from fetuses with severe growth retardation. The number of patients sampled is given in each instance. Results are given as means (SE). The Student's *t*- and Mann–Whitney tests were used for statistical analysis.

Results

Groups 1 (caesarean section for fetal indication) and 2 (for maternal indication) were well matched for birthweight and gestational age (Table 1). In both groups there were more small-for-gestational-age infants than expected. It was anticipated that group 1 would be more severely affected, and this was confirmed in that more babies weighed less than the 10th centile (90% compared with 70%, P = 0·025 Fisher's exact test).

The intervals to delivery, from the times of induction of general anaesthesia, the skin incision and the uterine incision were measured in all cases. The operative procedure was somewhat slower in group 3, but the times from uterine incision to delivery were short and similar in all groups, irrespective of general anaesthesia or epidural analgesia.

Fetal movements and heart rate analysis

The number of fetal movements recorded per hour was significantly less in group 1 (fetal indication) than in group 2 (maternal indication). The basal heart rate was similar in groups 1 and 2 (at 32 weeks mean gestational age) but was less in group 3 (at 39 weeks), ascribable to the fall in basal heart rate with increasing gestational age (Visser *et al.* 1982) (Table 2).

The variation in FHR (mean range) was above average for age in group 3 (58·1 SE 3·3 ms: normal mean 48·9 SE 2·8 ms, Dawes *et al.* 1985), but low in group 2 (35·0 SE 2·5 ms: normal 44·4 SE 1·5 ms at 32 weeks) and grossly reduced in group 1 to 20·6 SE 1·2 ms (54% of normal). Nine fetuses in group 1 but none in group 2 had a mean range of ≤20 ms but there was thus some overlap between the two groups with respect to FHR variation in the range 20–30 ms. In group 1 the numbers of accelerations were significantly reduced compared with group 2 and there were significantly more decelerations. Table 2 also shows that the number of minutes of high variation (a better measure of reactivity than accelerations, especially before 34 weeks) was grossly reduced in group 1.

The values of baseline variation (35·0 (3·0), 38·7 (2·6), and 39·2 (1·8) ms in groups 1–3 respectively) were similar, confirming that this measure of long-term heart rate variation (the lowest frequency, <1/10 min) does not reflect fetal health (Dawes *et al.* 1985).

Umbilical blood gas measurements

The umbilical artery $P\text{O}_2$ was significantly lower in group 1 than in group 2, and significantly lower in group 2 than in group 3. There was a large reduction in the calculated oxygen saturation of blood supplied to the fetal tissues in group 1 (Table 3).

There was little evidence of metabolic acid-

Table 1. Birthweight and gestational age of the three groups of patients classified by reasons for delivery by caesarean section

	Group 1 (fetal indication) (n = 21)	Group 2 (maternal indication) (n = 20)	Group 3 (other) (n = 30)
Gestation (weeks)	31·9 (0·6)	32·3 (0·6)	38·6 (0·1)*
Birthweight (g)	1233 (111)	1482 (107)	3162 (84)*
Birthweight centile			
>25th	0	2	22
10–25th	2	4	7
3–10th	11	8	1
<3rd	8	6	0

*P<0·001 compared with either group 1 or group 2 (Student's t-test).
Results are mean (SE) values.

Table 2. Analysis of fetal movement and heart rate by group of patient delivered by caesarean section for different indications

	Group 1 (fetal indications) (n = 21)	P*	Group 2 (maternal indications) (n = 20)	P*	Group 3 (other) (n = 30)
Fetal movements/h	11·7 (2·2)	<0·01	44·0 (9·1)		78·4 (15)
Basal heart rate (beats/min)	143·5 (2·3)		139·6 (2·0)	<0·01	131·7 (1·6)
FHR variation					
Mean range (ms)	20·6 (1·2)	<0·001	35·0 (1·5)	<0·001	58·1 (3·3)
Number of accelerations					
>10 beats/min and 15 s	2·0 (0·5)	<0·01	8·0 (1·6)	<0·001	20·2 (1·5)
>15 beats/min and 30 s	0·5 (0·2)	<0·05	3·9 (1·3)	<0·001	15·7 (1·4)
Number of decelerations					
>10 beats/min and 60 s	0·6 (0·2)	<0·01	0·05 (0·05)		0·2 (0·1)
>20 beats/min and 30 s	1·9 (0·3)	<0·001	0·3 (0·2)		0·3 (0·2)
Min of high variation	4·2 (1·2)	<0·001	24·4 (3·0)	<0·001	40·3 (1·9)

*Significance of difference between groups 1 and 2 and between groups 2 and 3 (Mann–Whitney test).
Results are means (SE) values.

aemia in any group as shown by the base deficits. But there was some respiratory acidaemia in groups 1 and 2, compared with group 3, with readings that were relatively lower (artery and vein) for blood pH and raised for Pco_2. When group 3 was divided into subgroups delivered under general anaesthesia or epidural analgesia, there were no differences in any measure of blood gas status.

In group 1 the mean umbilical artery Po_2 of the nine fetuses with a mean range of <20 ms was no different (6·5 SE 1·2 mmHg) from the 12 fetuses with higher FHR variation (5·9 SE 0·8 mmHg). But in group 2, the 11 fetuses with a mean range of >30 ms had a significantly higher artery Po_2 (11·8 SE 1·3 mmHg) than the others in the group with a mean range of 20–30 ms (7·8 SE 1·2 mmHg; P<0·05 Student's t-test). When groups 1 and 2 were combined there was a significant correlation between the mean minute range of heart rate variation and umbilical artery Po_2 (r = 0·54, P = 0·0003: and see Fig. 1). A similar correlation was not seen within group 3. The individual readings of the umbilical artery Po_2 and mean range of the fetal heart rate are plotted in Fig. 1 together with the mean ± SE values. The extent of the overlap between the groups is shown.

Other blood and amniotic fluid measurements

There were no significant differences between groups in blood lactate concentrations, consistent with the lack of fetal metabolic acidaemia.

Table 3. Umbilical cord blood gas values in groups of patients by reasons for delivery

	Group 1 (fetal indication) (n = 21)	P*	Group 2 (maternal indication) (n = 20)	P*	Group 3 (other) (n = 30)
Umbilical artery					
pH	7·23 (0·01)		7·26 (0·01)		7·27 (0·01)
Po_2 (mmHg)	6·1 (1·6)	<0·001	10·0 (1·0)	<0·05	13·0 (0·9)
Pco_2 (mmHg)	58·4 (1·7)		55·8 (1·0)	<0·001	49·7 (1·2)
Base deficit (mmol/l)	5·2 (0·6)		4·0 (0·4)		4·6 (0·3)
Umbilical vein					
pH	7·27 (0·01)		7·29 (0·01)	<0·001	7·33 (0·01)
Po_2 (mmHg)	16·9 (1·1)		20·1 (1·4)	<0·01	25·9 (1·2)
Pco_2 (mmHg)	47·7 (1·7)		46·4 (1·1)	<0·001	40·0 (0·8)
Base deficit (mmol/l)	5·5 (0·4)		4·5 (0·4)		4·8 (0·3)

*Significance of difference between groups 1 and 2 and between groups 2 and 3 (Student's t-test).
Results are mean (SE) values.

Plasma glucose concentration was reduced in group 1 compared with group 3, and the blood concentration of the gluconeogenic amino acid alanine was significantly increased in group 1. The latter correlated negatively with the mean minute range of heart rate variation (r = 0·53, P = 0·004; groups 1 and 2 combined) (Table 4).

The amniotic fluid erythropoietin values were highest in group 1 and lowest in group 3; the differences were significant between all three groups. The values of groups 1 and 2 combined were inversely related to umbilical artery Po_2 (r = 0·52, P = 0·014). There were no significant trends in the concentrations of hypoxanthine, xanthine, uridine or urate between groups (Table 4).

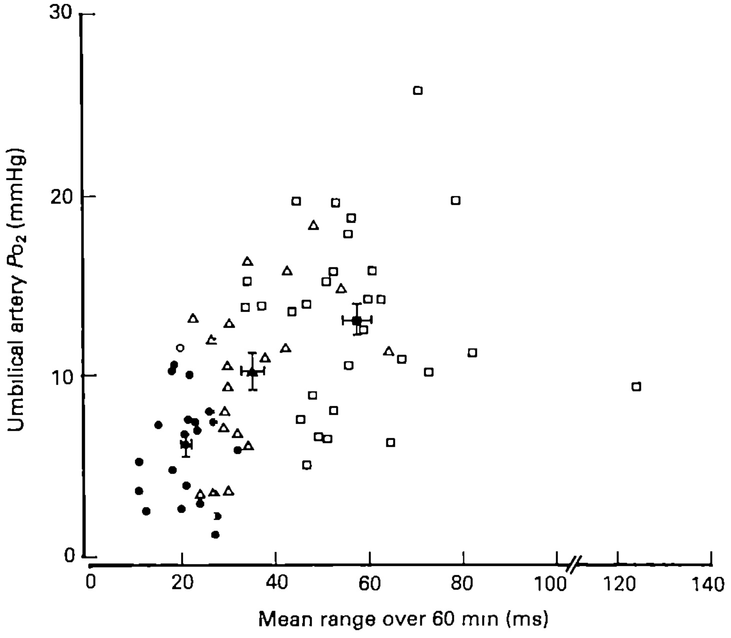

Fig. 1. The relation between the umbilical artery Po_2 at delivery and the mean range of the fetal heart rate before delivery. ●, Group 1; △, group 2; □, group 3. The only fetus in group 1 without decelerations is shown as ○. ┼ ┼ ┼ denote the means and standard errors for groups 1, 2 and 3 respectively.

Table 4. Comparison of some indices of asphyxia between groups of patients delivered by caesarean section

	Group 1 (fetal indication)	P^*	Group 2 (maternal indication)	P^*	Group 3 (other)
Umbilical artery					
β-endorphin (ng/l)	101 (15·7) (n = 7)	<0·05	59 (11·4) (n = 7)	<0·001	136 (13·7) (n = 16)
Glucose (mmol/l)	2·9 (0·2) (n = 11)		3·5 (0·3) (n = 17)		4·3 (0·3) (n = 26)
Lactate (mmol/l)	3·5 (0·6) (n = 11)		2·4 (0·2) (n = 17)		2·4 (0·03) (n = 26)
Alanine (mmol/l)	0·5 (0·1) (n = 11)	<0·01	0·3 (0·02) (n = 17)	<0·05	0·4 (0·03) (n = 26)
Umbilical vein					
β-endorphin (ng/l)	135 (22·7) (n = 11)	<0·05	75 (5·3) (n = 16)	<0·01	114 (12·8) (n = 18)
Amniotic fluid					
Erythropoietin (mu/ml)	66 (14·8) (n = 7)	<0·05	23 (4·1) (n = 14)	<0·05	15 (1·8) (n = 14)
Hypoxanthine (μmol/l)	2·4 (0·9) (n = 7)		1·6 (0·3) (n = 14)		1·6 (0·2) (n = 14)
Xanthine (μmol/l)	3·7 (0·8) (n = 7)		2·8 (0·3) (n = 14)		2·7 (0·2) (n = 14)
Uridine (μmol/l)	1·2 (0·3) (n = 7)		1·4 (0·2) (n = 14)		1·1 (0·05) (n = 14)
Urate (μmol/l)	166 (23) (n = 7)	<0·05	233 (17) (n = 14)		197 (7·5) (n = 14)

*Significance of difference between groups 1 and 2 and between groups 2 and 3 (Mann–Whitney test).
Results are mean (SE) values, n denotes the number of samples.

Table 5. Observations on two asphyxiated infants born by elective caesarean section

	Infant 1	Infant 2
Umbilical artery		
pH	6·985	6·982
$P\mathrm{o}_2$ (mmHg)	2·2	3·3
$P\mathrm{co}_2$ (mmHg)	90·5	114·7
Base deficit (mmol/l)	−13·8	−14·2
Lactate (mmol/l)	19·9	—
Glucose (mmol/l)	0·62	—
Alanine (mmol/l)	1·5	—
β-endorphin (ng/l)	—	>1500
Umbilical vein		
β-endorphin (ng/l)	892	1225
Amniotic fluid		
Erythropoietin (mu/ml)	—	>400

Fetal asphyxia

It was not possible to study fetal asphyxia systematically. However, we obtained samples from two patients admitted with absent fetal movements and 'terminal' (Visser & Huisjes 1977) FHR traces. The protocol of delivery and collection of samples was identical to that of the study groups, and the results are shown in Table 5. Both infants survived, both with evidence of neurological damage.

Thus, with extreme hypoxaemia there was severe respiratory and metabolic acidaemia but also marked hypoglycaemia and hyperalaninaemia. Beta-endorphin levels were grossly elevated, as was the amniotic fluid concentration of erythropoietin.The scale of these changes is very large, when compared to the small differences between groups 1, 2 and 3.

Discussion

The classification of the reason for preterm delivery in groups 1 and 2 was made antenatally, solely on clinical grounds and was not influenced by postpartum measurements. The two groups were comparable with respect to maternal complications and drug use, as well as gestational age. Although the fetal heart rate records were, at worst, suboptimal, the pregnancies in group 2 were complicated by pre-eclampsia severe enough to justify delivery at a mean gestational age of 32 weeks. Under these circumstances the fetuses would not be expected to be normal, but to suffer from similar problems to those experienced in group 1. The comparison between groups 1 and 2 is not, therefore, between normal and abnormal, but between compromised fetuses with and without marked fetal heart rate abnormalities. Any differences between the groups can be related to the differences in FHR patterns, which were also numerically analysed.

Interpretation of the umbilical cord gas values must take into account the maternal pre-oxygenation, the type of anaesthesia and the method of caesarean section. For example, administration of 100% oxygen to healthy women before caesarean section under epidural anaesthesia caused an increase in umbilical artery and vein Po_2 and oxygen saturation, with no effect on pH, Pco_2 or base deficit (Young *et al.* 1980). In our study the umbilical artery Po_2 and oxygen saturation in groups 1 and 2 were lower despite maternal pre-oxygenation. The cord gas values were not affected by the type of anaesthesia (or epidural analgesia), consistent with previous reports (Hollmen *et al.* 1978; Downing *et al.* 1979). Fetal acidaemia may result if the time between uterine incision and delivery is prolonged (Crawford *et al.* 1976; Downing *et al.* 1979), but there were no differences between our three groups.

For these reasons we believe that the umbilical cord blood gas values reflect the fetal respiratory status before delivery. The measurements showed that the fetuses in groups 1 and 2 were hypoxaemic, but did not have a major degree of metabolic acidaemia. The other measurements that we made were all consistent with this conclusion. The raised erythropoietin concentration in the samples of amniotic fluid from groups 1 and 2 is evidence that the hypoxaemia was prolonged, not merely the result of the processes of delivery. Fetal plasma erythropoietin is increased with experimental hypoxia (Zanjani *et al.* 1974; Meberg 1980; Widness *et al.* 1982) and in situations with a high risk of intrauterine hypoxaemia including pre-eclampsia (Finne 1966; Thomas *et al.* 1983). Because amniotic fluid erythropoietin is derived from fetal urine, raised levels are unlikely to be the consequence of events at delivery. However, we have not excluded the possibility that the change might result from a reduced amniotic fluid volume.

The fetuses of group 1 were hypoglycaemic compared with group 3, and had higher alanine concentrations than those in either group 2 or 3. These changes are appropriate to their greater hypoxaemia, and similar to those of experimental growth retardation (Robinson *et al.* 1979).

Hypoxia *in utero* depletes the stores of liver glycogen (Shelley 1969) and predisposes to neonatal hypoglycaemia (Lubchenko & Bard 1971). Hypoglycaemia has been observed in growth-retarded human fetuses antenatally (Soothill *et al.* 1987), in labour (Phillips *et al.* 1968) or at birth (de Leeuw & de Vries 1976), as well as in fetal lambs with experimental growth retardation (Robinson *et al.* 1979). In fetal lambs hypoglycaemia causes hypoinsulinaemia (Robinson *et al.* 1980), which stimulates increased protein catabolism and amino acid oxidation (Simmons *et al.* 1974; Bassett & Madill 1974; Van Veen *et al.* 1983), and plasma alanine is increased (Robinson *et al.* 1979; Mestyan *et al.* 1975). Hyperaminoacidaemia also occurs in acutely asphyxiated newborn infants (Schultz *et al.* 1981) and acidotic adults (Marliss *et al.* 1972). Alanine accounts for a large part of the increment in circulating amino acids because lactic acid interrupts the glucose-alanine cycle by inhibiting hepatic uptake of alanine.

Although the fetuses of group 1 were hypoxaemic they were not asphyxiated as judged by the normal levels of blood lactate and base excess. This conclusion was confirmed by the normal amniotic fluid levels of adenine nucleotide metabolites indicating that ATP regeneration was not impaired. A fall in ATP concentration in human tissue increases concentrations of hypoxanthine, the central intermediate in purine metabolism, inside (Simmonds *et al.* 1982) and outside cells (Harkness & Lund 1983; Harkness *et al.* 1983). Our amniotic fluid data do not exclude some ATP depletion occurring less than about 30 min before delivery. Such rapid changes are reflected in cord blood (O'Connor *et al.* 1981) but can be obscured by pre-oxygenation during delivery. Furthermore, the acute effect of asphyxia is to increase the release of beta-endorphins as demonstrated in the two infants with birth asphyxia who had severe hypoxaemia with metabolic acidaemia, severe hypoglycaemia and hyperalaninaemia. The plasma levels of beta-endorphin-like immunoreactivity were much higher than in groups 1 and 2. The beta-endorphin is probably released from the pituitary, where ACTH and beta-endorphin are derived from the precursor pro-opiomelanocortin (Guillemin *et al.* 1977). Whereas the placenta can synthesize endorphin-like peptides (Liotta *et al.* 1982), the small umbilical arterio-venous differences in all three groups suggest that the placenta was not a major source. The small, but statistically significant, increase in the endorphin levels in group 1 compared with group 2 is consistent with the effect of hypoxia in fetal lambs (Wardlaw *et al.* 1981) or human newborn infants (Sankaran *et al.* 1983). The differences between groups 2 and 3 suggest that fetal plasma beta-endorphin levels may rise with increasing gestational age.

That 'decelerative' or 'suboptimal' fetal heart rate traces in group 1 are not associated with acidaemia confirms earlier observations (Visser *et al.* 1980; Henson *et al.* 1983). Their association with hypoxaemia agrees with the report of Bekedam *et al.* (1987).

The differences between the fetuses in groups 1 and 2 were, in terms of blood and amniotic fluid measurements, ones of degree. It is impossible to determine from these data which, if any, determined directly or indirectly the more pronounced abnormalities of the fetal heart rate including the decelerative patterns. The effects of hypoxaemia and hypoglycaemia on myocardial function need to be considered; possibly the reduced FHR variation is caused, at least in part, by direct myocardial depression. Central nervous control of FHR variation may also be relevant through release of an $\alpha 2$ adrenergic agonist, because in fetal lambs clonidine causes loss of FHR variation (without a change in basal heart rate) and an arrest of breathing and movements (Bamford *et al.* 1986).

Finally, the results demonstrate that measurement of FHR variation over 60 min can help identify fetuses which are becoming hypoxaemic, without acidaemia. The discrimination was, on average, good although there was overlap between groups 1 and 2. However, the patients in group 2 were not normal; and moreover the subset with a low FHR variation were as hypoxaemic as those from group 1 with a similar mean range. No measure of the FHR pattern would be expected to correspond exactly to the fetal blood gas status at delivery. The association was detected despite the interval between FHR analysis and blood sampling, and the events of delivery itself. Other factors, for example, fetal drug exposure or lesions of the central nervous system, could independently affect the FHR patterns and confuse their interpretation. However, unlike cordocentesis (Soothill *et al.* 1986) the technique is non-invasive and can be repeated many times in the same individual—a valuable attribute for the serial assessment of the compromised fetus. Our results suggest that a mean range <20 ms is abnormal, and of >20–30 ms is probably abnormal, especially if associated with decelerations.

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Classical versus low-segment transverse incision for preterm caesarean section: maternal complications and outcome of subsequent pregnancies

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Summary. In a retrospective, controlled, follow-up study of 326 women who had a primary preterm caesarean section, the risks of postoperative maternal morbidity and uterine rupture or dehiscence in subsequent pregnancies were investigated in relation to the mode of incision (classical compared with low-segment transverse incision). The classical incision was associated with a higher frequency of postpartum fever in the immediate postoperative period (16% compared with 6%, $P<0{\cdot}01$). Of the 326 patients reviewed 286 (88%) were contacted for information about subsequent pregnancies. Information was obtained for 70 pregnancies subsequent to a classical caesarean section, and 71 pregnancies subsequent to a low-segment transverse caesarean section, which had continued for more than 20 weeks gestation. Of the pregnancies after the classical operation 13% had abnormal scars compared with none of those after the low-segment transverse operation ($P=0{\cdot}0014$). The frequency of scar dehiscence was 6% after a classical scar compared with none after a low-segment transverse scar ($P=0{\cdot}0581$).

Over the past 10 years, the outcome for the preterm fetus has improved substantially; there has also been an increased use of caesarean section for the delivery of the preterm fetus. The preterm uterus frequently has a poorly developed lower uterine segment. The birth of the fetus through a low-segment transverse incision is at times difficult, especially where the presentation is other than vertex; and therefore many authors have advocated the use of the vertical uterine incision for these caesarean births (Keeping *et al.* 1980; Westgren & Paul 1985; Haesslein & Goodlin 1979).

Other authors have suggested that as the vertical incision has not been shown to improve fetal or neonatal outcome, it should not be used, as the low-segment transverse incision is probably safer for the mother (Schutterman & Grimes 1983).

It has also been suggested that a curved 'transverse' incision can give as much room as a vertical incision (Jovanovic 1985).

The risks of maternal morbidity and uterine rupture in subsequent pregnancies after classical caesarean section have been reported previously. But most of these studies were made before 1960 when caesarean section was reserved primarily for the term fetus. Peri-operative care has improved substantially since that time.

The low-segment transverse (LST) incision in

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the preterm uterus may involve the upper segment or require an extension in a '⊥' or 'J' fashion. This may result in a higher risk of maternal morbidity and uterine rupture in subsequent pregnancies than the LST incision in the term uterus.

The maternal risks associated with the classical and LST incisions in the preterm uterus at the present time are not known.

Women's College Hospital (WCH) is a tertiary care, referral, perinatal facility, providing service to the central-east region of Ontario, Canada. Many patients have a preterm delivery at WCH, but are cared for in subsequent pregnancies by other physicians and at other institutions.

Over the past 10 years there has been a tendency at WCH to use the classical incision for the caesarean birth of the preterm fetus, particularly if the presentation is other than vertex and the lower uterine segment is poorly developed.

In this study we wished to determine whether postoperative maternal morbidity and uterine rupture or dehiscence in subsequent pregnancies were greater after the classical incision for preterm caesarean section than after the low-segment transverse incision.

Subjects and methods

We reviewed the records of all patients who had had a primary caesarean section, without a simultaneous sterilizing procedure, for the birth of an infant(s) at 24–35 weeks gestation, between 1 January 1978 and 31 March 1984. Patients in whom the caesarean section was through a vertical uterine incision formed the study subjects (classical group). We did not differentiate between the classical and low vertical incisions, as any vertical incision in a preterm uterus is very likely to involve the upper segment. In all these women, the classical incision was used to decrease the risk of traumatic delivery and hopefully to improve the outcome for the fetus and neonate. Each study subject was matched with a control subject who was the next patient, within a 12-month period, who had been delivered after a low-segment transverse incision (LST group) and, when possible, fell within the gestational age ranges of 24–27 weeks, 28–31 weeks and 32–35 weeks. Within the controls were included patients who had required '⊥' or 'J' extensions to their low-segment transverse incision. This was considered appropriate as these extensions are complications of the low-segment transverse incision.

Maternal baseline data, as well as maternal and fetal and neonatal outcomes, were collected. Subjects and controls were contacted between 1 November 1985 and 30 June 1986 and asked to respond to a questionnaire detailing the number and timing of subsequent pregnancies, the outcome of those pregnancies and, if there were none, the reason for this. Physicians were also asked to complete and return a questionnaire regarding the outcome of these pregnancies. When questionnaires were incomplete, or when subsequent pregnancies were delivered at WCH, operative or delivery notes and hospital discharge summaries were reviewed.

It was estimated that a study with 78 patients per group would have an 80% chance of detecting a 10-fold reduction in risk of uterine dehiscence or rupture, from 10% to 1%. This was based on the risk of uterine dehiscence and rupture in previous studies following the classical and low-segment transverse caesarean sections.

The tests of statistical significance used were χ^2 for proportions (using Yates' correction when there was only 1 degree of freedom) and Fisher's exact test (one-tailed) for differences in the frequency of scar dehiscence and abnormal scars.

The level of statistical significance was set at $P = 0{\cdot}05$.

Results

There were 163 patients in the classical group and 163 patients in the LST group. The groups were comparable with respect to antenatal transfers, antenatal complications, admission haemoglobin, gestational age at delivery, general anaesthesia and use of prophylactic antibiotics. Patients in the classical group were of higher parity and more likely to be over 30 years of age at the time of the caesarean section, than those in the LST group. Fetal presentation was much more likely to be breech or transverse lie in the classical group (76%) compared with the LST group (32%) ($P \ll 0{\cdot}001$) (Table 1). In the LST group there were seven patients who required an extension of the uterine incision in either a '⊥' (six patients) or 'J' (one patient) fashion.

Postpartum (maternal) fever, as defined by a temperature of >38 °C on two occasions, 24 h apart, occurred significantly more often after a classical caesarean section (16% *vs* 6%,

Table 1. Maternal and fetal characteristics in two groups of women delivered by caesarean section

	Classical (n = 163)		LST (n = 163)	
Characteristics	n	(%)	n	(%)
Maternal age >30 years	63	(39)	43	(26)*
Parity				
0	77	(47)	95	(58)**
1	46	(28)	48	(30)
≥2	40	(25)	20	(12)
Antenatal transfer	129	(79)	121	(74)
Antenatal complication†				
Hypertension	31	(19)	42	(26)
Diabetes	3	(2)	9	(6)
Antepartum haemorrhage	48	(29)	44	(27)
Premature rupture of membranes	72	(44)	84	(52)
Preterm labour	92	(56)	95	(58)
Chorioamnionitis	25	(15)	20	(12)
Intrauterine growth retardation	27	(17)	18	(11)
Multiple pregnancy	20	(12)	25	(15)
Rupture of membranes >24 h	63	(39)	58	(36)
Admission haemoglobin <10 g/dl	13	(8)	9	(6)
General anaesthesia	123	(75)	131	(80)
Use of prophylactic antibiotics	40	(25)	36	(22)
Gestational age at delivery (weeks)				
24–27	49	(30)	38	(23)
28–31	85	(52)	89	(55)
32–35	29	(18)	36	(22)
Fetal presentation‡				
Breech or transverse lie	124	(76)	52	(32)***

*$\chi^2_1 = 5{\cdot}05$, $P<0{\cdot}025$; **$\chi^2_2 = 8{\cdot}59$, $P<0{\cdot}025$; ***$\chi^2_1 = 62{\cdot}25$, $P \ll 0{\cdot}001$.

† One patient may have one or more complications

‡ First fetus in the case of multiple pregnancies.

Table 2. Postoperative complications

	Classical (n = 163)		LST (n = 163)	
Complication	n	(%)	n	(%)
Blood loss ≥1000 ml or need for blood transfusion	32	(20)	27	(17)
Postpartum fever	26	(16)	10	(6)*
Postpartum endometritis	13	(8)	6	(4)
Sepsis	10	(6)	3	(2)
Wound infection/dehiscence	2	(1)	5	(3)
Paralytic ileus	2	(1)	1	(<1)
Thromboembolism	—	—	2	(1)
Other	2	(1)	2	(1)
One or more of the above complications	57	(35)	44	(27)
Postnatal hospital stay (days)				
≥7	109	(67)	116	(71)
≥14	3	(2)	5	(3)

* $\chi^2_1 = 7{\cdot}03$, $P<0{\cdot}01$. (When adjusted for parity $\chi^2_{\mathrm{mH}} = 5{\cdot}82$, $P<0{\cdot}025$; when adjusted for maternal age $\chi^2_{\mathrm{mH}} = 6{\cdot}30$, $P<0{\cdot}025$.)

$\chi^2 = 7{\cdot}03$, $P<0{\cdot}01$) than after an LST caesarean section; this difference remained significant when adjusted for parity ($\chi^2_{mH} = 5{\cdot}82$, $P<0{\cdot}025$) and for maternal age ($\chi^2_{mH} = 6{\cdot}30$, $P<0{\cdot}025$). Blood loss ≥1000 ml or need for blood transfusion, endometritis, sepsis, wound infection or dehiscence, paralytic ileus, and thromboembolism were not significantly different between the two groups, although there was a trend towards infectious complications in the classical group (Table 2). There was no difference in postnatal hospital stay (Table 2).

There were no differences in perinatal mortality or low Apgar scores (<7 at 1 and 5 min) between the two groups.

The number of patients who were contacted at the time of the study was 146 (90%) in the classical group and 140 (86%) in the LST group. The maternal response rate from those located was very high (96–97%). There were 77 patients in the classical group and 76 patients in the LST group with no subsequent pregnancies. For most of these patients, the reason for this was voluntary (67 patients in the classical group and 54 in the LST group stated they wished no further pregnancies).

Information was available for 86 pregnancies subsequent to a classical caesarean section and 90 pregnancies after a low-segment transverse caesarean section. The pregnancy loss before 20 weeks gestation was 18% and was similar in the two groups: (17% in the classical group and 20% in the LST group).

The frequency of preterm delivery was high but similar in both groups (18% of all pregnancies, 22% of pregnancies >20 weeks).

The details (as supplied by the physicians) of the outcome of subsequent pregnancies which went beyond 20 weeks gestation, are outlined in Table 3. There were 70 pregnancies with a previous classical scar and 71 pregnancies with a previous low-segment transverse scar. Delivery and operative notes were reviewed by the authors for 44% of the pregnancies following a classical caesarean section and for 56% of those following a low-segment transverse caesarean section.

The prevalence of labour before the caesarean section was similar in both groups. Delivery was more likely to be by repeat caesarean section, however, if there was a classical scar than if there was a low-segment transverse scar (97% *vs* 83%, $P<0{\cdot}025$). There were four pregnancies complicated by uterine dehiscence in the classical group compared with none in the LST group ($P = 0{\cdot}0581$) (Table 3). These four, which were labelled as 'dehiscence', were described in the delivery/operative note or on the questionnaire as follows: (i) '. . . small uterine rupture about 1 cm in diameter . . .'; (ii) '. . . scar was so thin that fingers could be seen through the scar . . . would not be able to tolerate another pregnancy . . .'; (iii) '. . . near rupture of old scar'; (iv) '. . . dehiscence requiring metroplasty . . . suggested hysterogram prior to next pregnancy . . .'.

In addition, there were five pregnancies where the scar was described as 'thin'. Overall, the scar was abnormal in nine pregnancies after classical caesarean section; there were no reports of abnormal scars after the LST incision ($P = 0{\cdot}0014$).

The characteristics of the pregnancies with an abnormal scar are outlined in Table 4. The numbers are too small to comment on the effect of postpartum fever or labour before the primary caesarean section on the condition of the scar.

There were no differences between the two groups in terms of perinatal mortality, birthweight <2500 g, and need for neonatal intensive care in subsequent pregnancies.

Discussion

Maternal postoperative morbidity associated with caesarean section continues to be much higher than that associated with vaginal delivery (Ott 1981). The most frequent complication is infection (particularly endometritis) and the next most frequent is the need for blood transfusion. Major, non-infectious complications associated with caesarean section such as paralytic ileus, pulmonary embolism and operative lower urinary tract injury are rare (Petitti 1985). The patients in our series of preterm caesarean section experienced a relatively high postoperative morbidity (31%). The risk of postpartum fever was higher if the incision used was a classical or vertical one than if a low transverse incision was used. Other factors which are known to affect the occurrence of infectious morbidity such as anaemia, general anaesthesia, prolonged rupture of the membranes, chorioamnionitis, preterm labour before caesarean section, and use of prophylactic antibiotics were evenly distributed between groups and therefore cannot explain the association found between the classical incision and postpartum fever. Although socio-

Table 3. Outcome of pregnancies >20 weeks gestation subsequent to preterm caesarean section

	Classical (n = 70)		LST (n = 71)	
Outcome	n	(%)	n	(%)
Labour before delivery				
Overall	20	(29)	28	(39)
Before caesarean section	18	(26)	16	(27)
Mode of delivery				
Vaginal	2	(3)	12	(17)*
Caesarean section	68	(97)	59	(83)
Scar				
OK or no comment	61	(87)	71	(100)
Abnormal	9	(13)	0 ***	(—)**
Dehiscence	4		0	—
Thin	5		0	—

Significance of difference between the two groups: *$\chi^2_1 = 6{\cdot}28$, $P<0{\cdot}025$; **Fisher's exact test $P = 0{\cdot}0014$; *** Fisher's exact test $P = 0{\cdot}0581$.

Table 4. Characteristics of nine women with abnormal scars in pregnancies subsequent to classical caesarean section

	At primary caesarean section				At subsequent caesarean section (CS)		
Patient	Maternal age (years)	Parity	Gestational age (weeks)	Postpartum fever	Labour before section	Gestational age (weeks)	Scar abnormality
1	29	0	26	Yes	No	38	Thin
2	29	0	26	Yes	No	38	Thin
3	25	0	27	Yes	Yes	39	Dehiscence
4	32	0	30	No	No	37	Dehiscence
5	33	1	31	No	Yes	37	Dehiscence
6	26	0	28	No	No	38	Dehiscence
7	24	0	26	No	No	36	Thin
8	24	0	26	No	Yes	36	Thin
9	22	0	31	No	No	38	Thin

economic status was not assessed, we would not expect it to differ between the two groups.

Maternal morbidity and mortality in subsequent pregnancies is dependent, to some degree, on the risk of scar rupture. This risk for the classical scar has been reported to vary from 2 to 6% (Case *et al.* 1971; Dewhurst 1956, 1957; Lawrence 1953; Morley 1961; Clow & Crompton 1973). In a comprehensive review, Dewhurst (1956) reported that the incidence of classical scar rupture was 2·2% overall but increased to 4·7% if patients went into labour and 8·9% if patients had a vaginal delivery. In a prospective study, Pedowitz & Schwartz (1957) found the incidence of classical scar 'silent' rupture to be 18·2%. These ruptures were all incidental findings at the time of operation.

The classical scar rupture, when it presents clinically, is usually an acute event and frequently occurs before labour and before term.

It has been associated with both high maternal (0–20%) and fetal mortality (50–100%) rates (Hindman 1948; Holland 1920; Dewhurst 1957; Lawrence 1953; Morley 1961; Lawson & Ajabor 1968; Wilson 1951).

When the low-segment transverse incision was introduced in the early 1930s (Case *et al.* 1971), the risk of uterine rupture in subsequent pregnancies varied from 0·2% to 0·7% (Dewhurst 1957; Lawrence 1953; Morley 1961; Lavin *et al.* 1982). When the low-segment transverse scar ruptures, it is less likely to be a complete rupture or clinically apparent, is more likely to occur during labour, and is rarely associated with maternal or fetal death.

In many studies there is no clear distinction made for the 'low vertical scar'. However, it appears that the risk of uterine rupture of the low-segment vertical scar lies somewhere in between that of the classical and the low-segment transverse scars (Palerme & Friedman 1966; Cox *et al.* 1982).

This study emphasizes that the risk of the classical incision is still present. Although the level of significance was set before the study at $P = 0{\cdot}05$, one can argue that the difference in the occurrence of uterine dehiscence ($P = 0{\cdot}0581$) is unlikely to be due to chance alone. That is, there is a true increase in the incidence of uterine dehiscence or rupture following the classical compared with the low-segment transverse caesarean section.

Patients undergoing classical caesarean section were more likely to be >30 years of age, multiparous and to have had postpartum fever.

It is not possible to determine the effect of these factors on uterine dehiscence because of the small number of subsequent pregnancies.

Some may argue that the reporting of scar abnormalities may differ between the two groups, either because physicians are more likely to look for a scar abnormality after a previous classical caesarean section or, once found, they are more likely to report it. Only a blinded prospective study would eliminate this problem.

The decision to perform a classical incision is related to two issues. The first is whether or not a classical incision will significantly improve fetal or neonatal outcome. The second is whether or not the operation places the mother, or her subsequent pregnancies, at increased risk.

Randomized controlled trials of sufficient size are urgently required to determine the best approach to the birth of the preterm fetus, i.e. the approach which will do more good than harm to both fetus and mother, not just in the current pregnancy, but also in subsequent pregnancies.

The results of this study support the continuing concern that the classical incision for caesarean section increases the risk of postoperative morbidity and abnormal scars in subsequent pregnancies compared with the low-segment transverse incision.

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Plasma corticotrophin-releasing factor (CRF) in normal pregnancy

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Summary. Corticotrophin-releasing factor (CRF) was measured directly in maternal plasma using an immunoradiometric assay (IRMA). In the first and second trimester CRF levels were within the non-pregnant range (mean 15 pg/ml). A total of 72 women was followed sequentially from 28 weeks until delivery and CRF levels rose from a median of 20 pg/ml at 28 weeks to 1320 pg/ml at 40 weeks and 1732 pg/ml during labour. There was a strong correlation ($r_s = 0{\cdot}81$, $P<0{\cdot}001$) between gestational age and CRF levels. The rate of rise of CRF (pg/ml) per week was associated with weight gain ($r_s = 0{\cdot}36$, $P<0{\cdot}05$) but with no other obstetric variable. There was an association between umbilical cord and maternal plasma CRF levels ($r_s = 0{\cdot}54$, $P<0{\cdot}01$).

The human placenta secretes many proteins and steroids into the maternal circulation and their measurement has been used to assess fetal well-being. The development of specific and accurate diagnostic tests for the identification of the fetus at risk of death or damage *in utero* has been an elusive goal. Consequently, recently identified placental proteins need to be investigated for their potential use in predicting abnormal pregnancy outcome. The placenta synthesizes a number of peptides resembling hypothalamic and pituitary hormones, both in structure and function, and during pregnancy the maternal plasma levels of these peptides may exceed that of non-pregnant subjects. The placenta has been identified as a source of the hypothalamic releasing hormones (RH), thyrotrophin RH, luteinizing hormone RH, growth hormone RH and corticotrophin-releasing factor (CRF) (Shambaugh *et al.* 1979; Siler-Khodr & Khodr 1978; Baird *et al.* 1985; Shibasaki *et al.* 1982) and of the pituitary hormones ACTH, β-endorphin, β-lipotrophin and α-melanocyte stimulating hormone (Rees *et al.* 1975; Liotta & Krieger 1980; Genezzani *et al.* 1981; Clark *et al.* 1978). There is also considerable homology between luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) (Closset *et al.* 1973) and between growth hormone (GH) and human placental lactogen (hPL) (Li *et al.* 1971).

CRF is a 41 amino acid peptide originally isolated from ovine hypothalami (Vale *et al.* 1981). It is released from the hypothalamus in response to stress and stimulates the release of ACTH from the anterior pituitary, which in turn stimulates cortisol secretion from the adrenal gland. Placental and extracted maternal plasma CRF

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are bioactive (Shibasaki *et al.* 1982; Linton *et al.* 1987) and maternal plasma levels are elevated in the third trimester of pregnancy, declining rapidly postpartum (Sasaki *et al.* 1984; Campbell *et al.* 1987). CRF is also detectable in umbilical cord blood at delivery (Campbell *et al.* 1987; Goland *et al.* 1986; Sasaki *et al.* 1987). The identification of CRF is of interest as ACTH has been isolated from the placenta (Rees *et al.* 1975) and there is a tendency for plasma ACTH levels to rise in the second and third trimester of pregnancy with a rapid rise in labour (Carr *et al.* 1981). Plasma total cortisol rises throughout pregnancy and rapidly during labour whereas unbound cortisol, the biologically active moiety, increases significantly only in the second and third trimester (Abou-Samra *et al.* 1984); but despite these elevated cortisol levels there is little evidence of hypercortisolism in pregnancy. It is not known what role CRF may play in the modulation of the maternal and fetal hypothalamo-pituitary-adrenal axis during gestation but Campbell *et al.* (1987) found no correlation between CRF and total cortisol during pregnancy and labour.

The present study is descriptive and was designed to determine the levels of plasma CRF during pregnancy and parturition in maternal and umbilical cord plasma and to investigate the relation between these plasma levels and various obstetric variables to determine its potential use as an indicator of obstetric outcome. The study also examined the relation between umbilical cord and maternal plasma CRF at delivery.

Patients and methods

Single estimations of plasma CRF were made in 20 women in each of the first and second trimesters, and sequential samples were taken from 72 women from 20 weeks until delivery although not all women had a sample taken every 2 weeks. All the women had a normal pregnancy with a spontaneous vaginal delivery at term (>38 weeks) of a baby weighing >2·5 kg. For each estimation, 2 ml of venous blood were collected in a heparinized tube and kept on ice until centrifuged at 4°C for 10 min. A 1-ml sample was snap frozen on solid CO_2 and stored at −20°C until assay.

Umbilical vein CRF levels were compared with maternal CRF levels in 24 deliveries. Cord vein blood was obtained by double clamping the umbilical cord near the fetal umbilicus and close to the vaginal introitus. This portion of cord was excised and venepuncture performed using an 18-gauge needle and the plasma dealt with as described for maternal plasma.

The Mann–Whitney *U*-test and Spearman's rank correlation were used to analyse the data. The distribution of CRF for each 2-week period of the third trimester was non-gaussian and highly skewed to the right, which was not corrected by logarithmic transformation of the data so that CRF levels were expressed in terms of medians and a cumulative frequency plot of CRF values given for each 2-week period. The rate of rise of CRF/week in individual patients was calculated, using simple linear regression slopes for a minimum of three antenatal values for each woman. To compare antenatal and labour values in the same woman the Wilcoxon one-sample rank test was used. Statistical analysis was performed using the statistical package MINITAB.

An immunoradiometric assay (IRMA) requiring no extraction step was used to measure CRF direct in plasma. It depends on the use of two antibodies directed to opposite ends of CRF-41 and consequently only intact molecules, and not fragments, are detected (Linton *et al.* 1987). A 200-µl sample of plasma was assayed directly for CRF 1-41 by overnight incubation with guinea-pig anti-(CRF 1-20) antiserum and ^{125}I-labelled rabbit anti-(CRF 36-41) immunoglobulins (IgG). Bound and free labelled IgG were separated using sheep anti-(guinea-pig Fc region) IgG coupled to a dynosphere solid phase. The sensitivity of this IRMA is 1 pg/ml.

Details of the patients' age, race, past obstetric history, blood group, sickle status, smoking habit, weight gain in pregnancy, weight loss at term and maternal weight were recorded along with details of the gestational age at the time of onset of labour, whether labour was spontaneous or induced and the length of the first stage, fetal sex, weight and placental weight.

Results

CRF values did not exceed the non-pregnant range (average 15 pg/ml) in the first and second trimesters. There was a wide range of values at each gestational age (Table 1) and CRF values rose dramatically in the third trimester from a median of 20 pg/ml at 28 weeks to 1320 pg/ml at 40 weeks (Fig. 1). The median value in labour

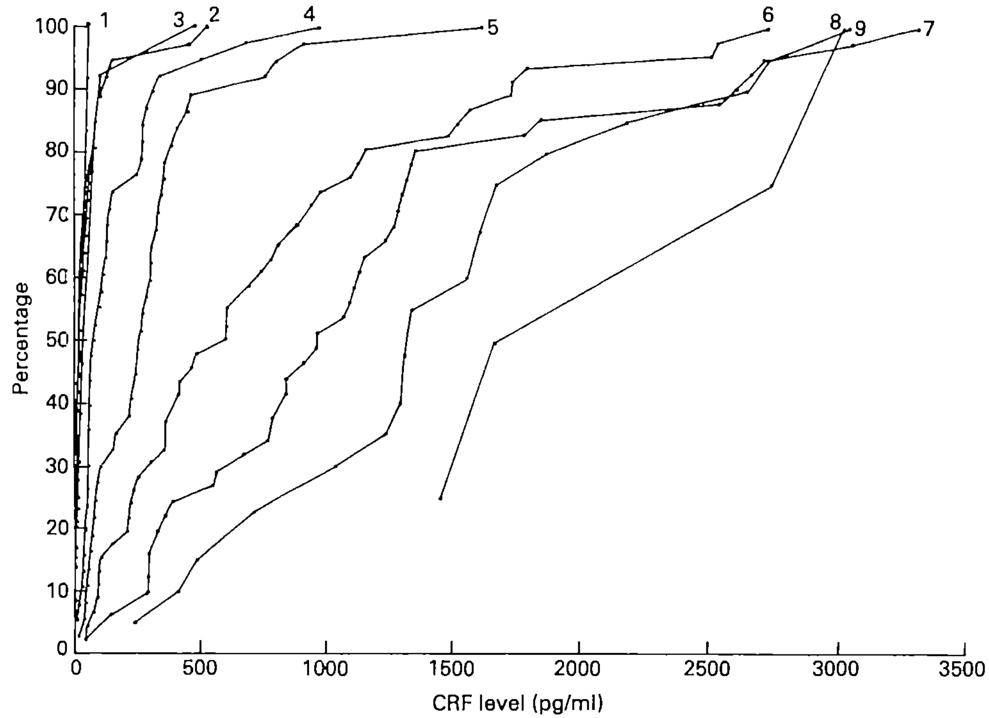

Fig. 1. Cumulative frequency plot of CRF values at each fortnight of gestation from (1) 20–26 weeks to (9) 42 weeks. ● represents one observation.

was 1732 pg/ml (Table 2), but there was no progressive rise during the course of labour. There was a strong correlation between gestational age and CRF levels in the third trimester ($r_s = 0·81$, $P < 0·001$). In 11 subjects followed sequentially, the last antenatal value and labour values were obtained within a few days of each other (38–42 weeks) and there was a significant difference between them ($P < 0·01$ (Wilcoxon test) median antenatal 1117 pg/ml; median labour 1849 pg/ml). Because of the wide range of CRF values at each gestational age the rate of rise of CRF (pg/ml) per week in individual subjects was calculated over the third trimester ($n = 48$). All maternal obstetric variables were examined against the weekly rate of rise of CRF with the only significant observation being that CRF levels correlated with maternal weight gain in pregnancy ($r_s = 0·36$, $P < 0·05$, Fig. 2).

The umbilical vein CRF values ranged from 8 to 237 pg/ml whereas the maternal CRF values ranged from 260 to 3564 pg/ml and there was a statistically significant correlation between them ($r_s = 0·54$, $P < 0·01$, Fig. 3).

Discussion

This is the first report of the sequential measurement of CRF in pregnancy and parturition in which statistical analysis shows a non-gaussian distribution of CRF values at each stage of gestation and that the calculation of medians is of potential use when defining the observed range in normal pregnancy. The sharp rise in CRF values to term is similar to that found for pregnancy-associated plasma protein A (PAPP-A) (Smith *et al.* 1979) but unlike most other placental proteins such as hPL and pregnancy-specific β_1-glycoprotein (SP1) which plateau in the late third trimester (Bishof *et al.* 1980). The small but significant rise in CRF levels between the end of pregnancy and labour is of interest with regard to the possible role of CRF in the process of parturition but more data are required to elucidate this point. Certainly the marked rise in plasma CRF during labour reported by Sasaki *et al.* (1987) is not confirmed by the present study. The lack of association between CRF and a number of clinical variables makes the interpretation of CRF values less complex. Unlike some studies with hPL, SP1 or PAPP-A, CRF does not correlate with placental weight (Saxena *et al.* 1969), fetal sex or weight (Letchworth *et al.* 1971) or parity (Bishof *et al.* 1980). A significant difference in CRF levels in subjects who gain weight excessively may indicate that an abnor-

Table 1. CRF values (pg/ml) from 20 to 42 weeks

Gestation (weeks)	Number of patients	CRF (pg/ml) Median	(Range)	10th–90th centile
20–26	12	15	(1–49)	3–48
28	36	20	(1–520)	4–121
30	13	37	(1–466)	17–68
32	38	78	(1–965)	34–306
34	37	260	(16–1600)	55–440
36	46	593	(44–2732)	95–1720
38	41	960	(44–3315)	295–2605
40	20	1320	(239–3040)	485–2649
42	4	2208	(1450–3022)	1660–2750

Table 2. CRF values (pg/ml) during labour at different cervical dilatations

Cervical dilatation (cm)	Number of patients	CRF (pg/ml) Median	(Range)	10th–90th centile
2	14	1772	(453–3186)	530–2660
3–5	12	1691	(226–3349)	407–1928
6–9	13	1675	(526–2756)	666–2335
10	21	1699	(83–2698)	306–2430

mal pregnancy state such as diabetes, pregnancy-induced hypertension with oedema or gross oedema alone may influence the CRF levels, and further analysis of CRF levels and weight gain needs to be undertaken.

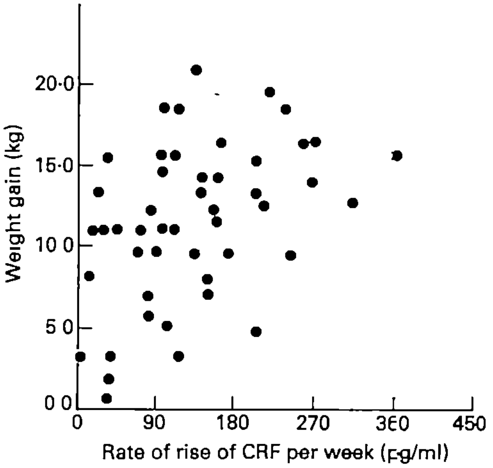

Fig. 2. Correlation between rate of rise of CRF (pg/ml) per week and maternal weight gain. ● represents one observation. $r_s = 0{\cdot}36$; $P<0{\cdot}05$.

There is a variation between investigators in terms of the absolute levels of CRF measured in the plasma and this may reflect the different assay and extraction systems used (Schulte & Healy 1985; Sasaki *et al.* 1984, 1987; Goland *et al.* 1986; Cunnah *et al.* 1985). The IRMA will only detect the CRF-41 molecule and requires no prior extraction whereas radioimmunoassay may also detect fragments of CRF and in most instances requires a prior extraction step. The data presented on CRF concentrations in umbilical cord plasma indicate that fetal CRF levels are only 3–4% of maternal levels. That does not support the observations made by Goland *et al.* (1986) who found greater CRF levels using an extracted RIA. Our data show a significant correlation (r_s = 0·54, $P<0{\cdot}01$) between umbilical vein CRF levels and maternal CRF levels which is in agreement with other groups (Goland *et al.* 1986; Sasaki *et al.* 1987) and may reflect a common source in the placenta. Unlike Sasaki *et al.* (1987) we have previously not been able to demonstrate a significant difference between umbilical artery and vein CRF levels, which one might expect with a peptide of placental origin, thus the CRF

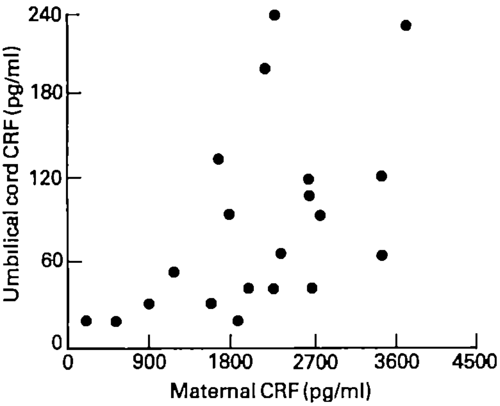

Fig. 3. Correlation between maternal CRF (pg/ml) and umbilical cord CRF (pg/ml) at delivery. ● represents one observation. $r_s = 0{\cdot}54$; $P < 0{\cdot}01$.

measured in umbilical cord samples may be fetal in origin and represent the normal circulating CRF in the fetus (Campbell *et al.* 1987).

The identification of another hypothalamic releasing hormone from the placenta may be an indicator of the totipotent nature of the cells of the placenta, but it is interesting to postulate that CRF may have a paracrine role in regulating protein expression and secretion from the placenta as has been suggested for LHRH and GRF (Siler-Khodr & Khodr 1978; Baird *et al.* 1985).

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Plasma corticotrophin-releasing factor (CRF) in abnormal pregnancy

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Summary. Maternal plasma levels of cortiocotrophin-releasing factor (CRF) have been measured in abnormal pregnancy states to assess their potential as biochemical markers for at-risk pregnancies. CRF levels were not significantly altered in patients with hydatidiform mole, polyhydramnios or diabetes. CRF levels were elevated in pregnancies complicated by accidental antepartum haemorrhage at 28 weeks ($P<0·03$) but not for the rest of the third trimester. In twin pregnancies CRF levels were significantly raised throughout the third trimester (28–32 weeks, $P<0·01$; 34–36 weeks, $P<0·001$). In patients with pregnancy-induced hypertension (28 weeks, $P<0·001$; 32–36 weeks, $P<0·001$; and 38–40 weeks, $P<0·01$), preterm labour and premature rupture of the membranes (28 weeks, $P<0·004$; 30–32 weeks, $P<0·002$; and 34–36 weeks, $P<0·001$), CRF levels were significantly raised and in some patients levels were elevated 11 weeks before the onset of signs or symptoms. These observations raise the possibility that maternal CRF measurement may be of use as a predictive indicator of certain at-risk pregnancies.

The assessment of fetal well-being includes both biochemical and biophysical approaches and although the former have proved disappointing, the latter have not provided the clinician with a test of high predictive value for detecting the compromised fetus. It is therefore important to assess recently identified placental peptides for their potential usefulness in predicting abnormal pregnancy states.

Corticotrophin-releasing factor (CRF) is a hypothalamic peptide released in response to stress which has been isolated from the placenta (Shibasaki *et al.* 1982) and reported by Wolfe *et al.* (1988) to be elevated in the third trimester of pregnancy, rising from 20 pg/ml at 28 weeks to 1320 pg/ml at term and 1732 pg/ml during labour. As CRF is not elevated above the normal non-pregnant range before 28 weeks gestation it would probably not be of use in the detection of first trimester abnormal pregnancy states but the finding of significantly low or high levels in the third trimester may indicate an at-risk pregnancy.

In this paper we report the levels of CRF in

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hydatidiform mole, twin pregnancy, pregnancies complicated by pregnancy-induced hypertension, preterm labour and premature rupture of the membranes, diabetes, antepartum haemorrhage and polyhydramnios.

Patients and methods

The observed range in normal pregnancy was constructed from a cohort of 72 normal subjects followed sequentially from 28 weeks to term (Wolfe *et al.* 1988). Three patients with hydatidiform moles were sampled before the uterus was evacuated. Fourteen twin pregnancies were sampled randomly (24 samples) and three of them were delivered preterm. Twenty-eight diabetic patients (14 insulin dependent and 14 diet controlled) were sampled sequentially during the third trimester (62 samples). A comparison of CRF levels in diet- and insulin-controlled diabetics and established and gestational diabetics was made. Four patients with accidental antepartum haemorrhage (11 samples) were also studied.

Sixty-three patients hospitalized with pregnancy-induced hypertension (blood pressure >140/90 mmHg on two occasions) were sampled on at least one occasion (103 samples). Ten of these patients had significant proteinuria. Of the original group of women sampled sequentially in the antenatal clinic seven developed pregnancy-induced hypertension. Twenty-one non-hypertensive women admitted in preterm labour or with ruptured membranes and who were delivered before 37 weeks contributed 39 samples; five of the women had been sequentially sampled from 28 weeks.

Seventeen samples were taken from patients with polyhydramnios. This group contained diabetics but most had no ascertainable reason for increased amniotic fluid.

The samples were assayed for CRF using a two-site immunoradiometric assay (IRMA) (Linton *et al.* 1987).

The distribution of CRF at each 2-week period of the third trimester was non-gaussian (Wolfe *et al.* 1988) and CRF levels are therefore expressed in terms of medians. The Mann–Whitney *U*-test was used to analyse the data. From the observed range in normal pregnancy the 90th centile was calculated.

Results

The CRF values in twin pregnancies (Table 1) were significantly higher than those in singleton pregnancies at each stage of gestation except at 38–40 weeks.

In all three hydatidiform moles, the maternal level of CRF was in the normal, non-pregnant range (15 pg/ml, 18 pg/ml, and 25 pg/ml). There was no significant difference between CRF levels in diabetics, gestational and established, and normal subjects (Table 1). Of the three samples taken in cases of accidental antepartum haemorrhage at 28 weeks there was a significant increase in CRF (Table 1) but not for the rest of the third trimester. There was no difference in CRF levels in the presence of polyhydramnios (Table 1).

The CRF values at all stages of pregnancy were highly significantly increased (Table 1) in patients hospitalized with pregnancy-induced hypertension, regardless of the severity of the disease. In the subjects followed sequentially through pregnancy, four had levels above the 90th centile as long as 11 weeks before the development of signs or symptoms of pregnancy-induced hypertension but the CRF values were within the normal range in three other subjects.

The CRF values in patients who went into preterm labour but with no signs of PIH, which might also elevate the CRF levels, demonstrated a highly significant increase at all stages of gestation (Table 1). Of the subjects followed sequentially through pregnancy, five went into preterm labour, four of them had levels above the 90th centile up to 11 weeks before the onset of preterm labour.

Discussion

The raised levels of CRF in twin pregnancies is in agreement with observations on other placental products (Nisbet *et al.* 1981; Spellacy *et al.* 1978; Towler *et al.* 1976) and may reflect the increased placental mass in twin pregnancy although we found no correlation between placental mass and CRF levels in singleton pregnancy. Diabetic patients have an increased perinatal and neonatal mortality and morbidity due to antenatal complications but this was not reflected in the CRF levels which showed no significant difference from the observed range in normal pregnancy, although the numbers studied were

Table 1. Median values of corticotrophin-releasing factor (CRF) (pg/ml) in twin pregnancies, diabetic pregnancies, pregnancies complicated by antepartum haemorrhage, polyhydramnios, pregnancy-induced hypertension (PIH) and preterm labour

	Gestational age (weeks)			
Patient group	<28	30+32	34+36	38+40
Normal pregnancies				
n	48	51	83	61
CRF (pg/ml)	20	60	351	1126
Twin pregnancies				
n	5	4	12	3
CRF (pg/ml)	87	505	1935	1597
Significance	<0·01	<0·01	<0·001	<0·4
Diabetic pregnancies				
n	15	18	26	61
CRF (pg/ml)	26	141	522	1126
Significance	1	<0·16	<0·6	<0·16
Antepartum haemorrhage				
n	3	5	3	
CRF (pg/ml)	133	60	493	
Significance	<0·03	<0·5	<0·7	
Polyhydramnios				
n	3	6	5	3
CRF (pg/ml)	15	153	307	706
Significance	0·95	0·25	0·9	0·75
Pregnancy-induced hypertension (PIH)				
n	14	23	45	23
CRF (pg/ml)	68	840	1253	1896
Significance	<0·001	<0·001	<0·001	<0·01
Preterm labour				
n	10	11	14	
CRF (pg/ml)	86	320	1198	
Significance	<0·001	<0·001	<0·001	

Results are median values of CRF.
n, Number of samples.
Significance shown is the difference from levels in normal pregnancies at the same gestational age

small. Similarly the numbers studied with an accidental antepartum haemorrhage were small and are difficult to interpret.

The initial observations on CRF levels in pregnancy-induced hypertension indicate that levels rise, which is like placental protein 5 (PP5) (Nisbet *et al.* 1981) and pregnancy-associated plasma protein-A (PAPP-A) (Lin *et al.* 1977) but unlike other placental proteins which are unchanged or decreased (Salem *et al.* 1983; Spellacy *et al.* 1971). It is not certain whether the rise in CRF is primary or secondary to the pregnancy-induced hypertension but CRF is a peripheral vasodilator (Fisher *et al.* 1983) and consequently the rise may be a compensatory response to pregnancy-induced hypertension.

The elevated levels of CRF in patients in preterm labour, and more importantly in patients some weeks before the onset of preterm labour, is of considerable interest as it raises the possibility of a clinical application in screening for, or predicting of, preterm labour. Darne *et al.* (1987) have reported elevated oestriol/progesterone ratios some weeks before spontaneous labour at term and 1–4 days before delivery in idiopathic preterm labour and, as they rightly state, any predictive test must be positive some weeks before delivery in order that preventive treatment can be initiated. Other placental proteins such as PP5 (Salem *et al.* 1981) have also been reported as raised in preterm labour but Westergaard *et al.* (1984), studying SP1, hPL and PP5, suggested that their measurement is unlikely to have any predictive value.

The biological function of placental proteins is not known (Chard & Klopper 1982) but their measurement has been used to try and assess fetoplacental function with varying degrees of success. After extraction, placental CRF has been shown to be bioactive but in the circulation it is bound to a carrier protein which may alter its potential activity (Linton & Lowry 1986), and its role in pregnancy, if any, has yet to be determined.

The finding of raised levels of CRF in the two abnormal pregnancy states which account for major causes of neonatal morbidity and maternal mortality is of importance. Further studies are required to assess plasma CRF levels in pregnancy, especially when there is a higher risk of preterm labour or PIH, in order that the predictive value of a CRF measurement for abnormal pregnancy states can be assessed.

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Massive feto-maternal haemorrhage: effect of passively administered anti-D in the prevention of Rh sensitization and haemolytic disease of the newborn

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Summary. Fourteen mothers carrying ABO compatible but Rh D incompatible pregnancies experienced large feto-maternal haemorrhages (estimated 188 ml, range 50–400 ml red cells). These were all treated with infusions of fresh frozen plasma containing anti-D at a mean immunoglobulin to fetal cell ratio of 129 i.u. (25·8 μg)/ml of fetal cells. At 48 h after the infusion 95% of fetal cells had been removed but 3 to 4 days were required for complete removal. The passively administered anti-D was detectable for up to 6–9 months but not after that time. Four of the 14 women had successful pregnancies subsequently although in two of these anti-C + D was present. Of these, one resulted in a clinically affected baby who survived exchange transfusion.

Routine administration of anti-D immunoglobulin following Rh D incompatible pregnancies has been shown to be highly effective for the prevention of active immunization and of haemolytic disease of the newborn. Failures of the conventional postnatal programme are largely attributable to administrative errors in providing therapy, to episodes of sensitization before delivery, or alternatively to feto-maternal haemorrhages that exceed the prophylactic capacity of the routine dose of anti-D immunoglobulin. The two former categories of failure should be amenable to improvements in organizational arrangements and to the introduction of antenatal therapy respectively. Massive feto-maternal haemorrhages will, however, continue to provide a potential cause for maternal immunization. These episodes must therefore be identified by routine examination of maternal blood after delivery or any other potentially sensitizing events. Although the prophylactic mechanism of passively administered anti-D remains uncertain there is evidence that high ratios of anti-D to Rh D-positive cells result in more rapid removal from the circulation (Mollison & Hughes-Jones 1967). Massive transplacental leaks (>50 ml) are rare (Mollison 1983) and little information is available about the expected success rate after an appropriately increased dose of anti-D.

Patients and methods

Fourteen Rh D-negative pregnant women with Rh D-positive ABO compatible fetuses were recorded as having experienced massive feto-maternal haemorrhages in South and Mid Wales during the period April 1982 to May 1986. Their clinical records are summarized in Table 1. Deliveries were managed in various hospitals throughout the region located between 5 and 112 miles from the transfusion centre. Incidents of major haemorrhage were reported at any time of the day or night and were handled by different staff on each occasion which made consistency in their management difficult. The transplacental

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Table 1. Clinical details of women with feto-maternal haemorrhage (FMH)

Patient no.	Probable cause of FMH	Maternal blood group	Baby's blood group	Kleihauer (fetal cells/lpf)	Immunoglobulin to fetal cell ratio (i.u./ml fetal cells)	Postnatal antibody screen	
						Months	Specificity
1	IUD 2 weeks antenatal	A Rh D neg.	Rh D pos.	50	168	3 6	Anti-D Nil
2	IUD 4 days antenatal	A Rh D neg.	Rh D pos.	400	133		
3	Forceps delivery	A Rh D neg.	A Rh D pos.	235	64	3 6 12	Anti-D Anti-D Nil
4	Normal delivery	O Rh D neg.	O Rh D pos.	230	59	22	Nil
5	APH, placenta praevia	A Rh D neg.	O Rh D pos.	50	230	6 9	Anti-D Nil
6	Retained placenta	A Rh D neg.	A Rh D pos.	150	283	3 6 14	Anti-D Anti-D Nil
7	Retained placenta	O Rh D neg.	O Rh D pos.	300	90	19	Nil
8	IUD 18 h antenatal	O Rh D neg.	O Rh D pos.	200	125	6	Nil
9	Normal delivery	AB Rh D neg.	AB Rh D pos.	100	162		
10	Normal delivery	A Rh D neg.	A Rh D pos.	90	120	9	Nil
11	Caesarean section	O Rh D neg.	O Rh D pos.	200	93		
12	Normal delivery	O Rh D neg.	O Rh D pos.	400	52·5	4 7	Anti-D Nil
13	APH, caesarean section	A Rh D neg.	A Rh D pos.	80	90	2 6	Anti-D Nil
14	Normal delivery	A Rh D neg.	O Rh D pos.	150	140	3 6 12	Anti-D Anti-D Nil

lpf, Low power field; APH, antepartum haemorrhage; IUD, intrauterine death

haemorrhage was estimated with the Kleihauer Beckte technique and, routinely, unusually high results were notified to the transfusion centre. Fetal cell counts were calculated according to standardized procedures (ACP Broadsheet 1978). On receipt of this information, the following protocol was instituted.

(i) Confirmation of maternal ABO and Rh D group and, wherever possible, determination of the newborn infant's ABO and Rh D group. (ii) Confirmation that the maternal serum lacked evidence of previous Rh D immunization. (iii) Performance of a Rh D group determination by the antiglobulin technique on the maternal blood sample. This was examined microscopically for mixed field agglutination as evidence for the presence of Rh D-positive fetal cells in the maternal circulation. (iv) When possible, clinical information about the infant's condition, cord blood haemoglobin and, if applicable, timing of intrauterine death was obtained.

Provided that the diagnosis of feto-maternal haemorrhage and Rh D incompatible pregnancy was verified, anti-D was administered at a dose, wherever possible, equivalent to 125 i.u. (25 μg) per ml of fetal cells estimated to be within the maternal circulation (WHO 1971). Because of the requirement for very large doses of anti-D, the assumed need to effect rapid cell clearance, and the expected greater efficacy of an intravenous preparation (Bowman 1982), fresh frozen plasma, as donated by regular anti-D plasmapheresis donors for immunoglobulin production, was supplied for administration by intravenous infusion. About 200 ml of plasma were transfused over 6 to 8 h. Routine clinical observations appropriate to blood transfusion were maintained, oral fluids were encouraged and the urine output monitored. Clinical staff were alerted for the possibility of symptoms of a mild haemolytic transfusion reaction particularly in view of the large quantity of incompatible cells requiring removal.

Results

Clinical details of the 14 pregnancies are shown in Table 1. The dose of anti-D administered varied from 52·5 to 283 i.u./ml fetal cells (mean 129 i.u./ml). This variation reflected the range of feto-maternal haemorrhages encountered as well as the availability of a suitable pack of frozen anti-D plasma for infusion.

Passively administered anti-D could be recovered in the maternal circulation in all women the following day. Because of the difficulties in collection of samples for assay and also ignorance of the maternal plasma volume, precise assessment of anti-D recovery could not be obtained. Fetal cell removal rates were rather variable (Fig. 1), 20% (range 0–69%) remaining at 24 h and 4·5% (range 0–16%) after 48 h. By 4–5 days all fetal cells appeared to have been removed. Eight of the 14 women had no side-effects during fresh frozen plasma infusion, three had slight rises in temperature, two experienced rigors and one had a severe allergic reaction so that the infusion was stopped. The dose of anti-D and the magnitude of incompatible cell removal did not appear to be a contributing factor.

The presence of anti-D during the first few months following infusion was assumed to reflect persistence of the original material. This eventually disappeared and in none of the women could anti-D be detected after 9 months. Four of the 14 women had a subsequent pregnancy, two did not develop anti-D although their babies were Rh positive. Of the other two who developed anti-D, one gave birth to a clinically unaffected Rh-positive infant and the other to an infant who survived after exchange transfusion.

Discussion

Massive feto-maternal haemorrhages are not commonly reported events and we have been

Table 2. Outcome of subsequent pregnancies in four women with massive feto-maternal haemorrhage (FMH) treated with anti-D

			Subsequent pregnancy				
Patient no.	Probable cause of FMH	Kleihauer (fetal cells/lpf)	Antenatal antibody screen	Cord blood groups	Direct antiglobulin test on cord cells	Haemolytic disease of the newborn	Survival of baby
1	IUD 2 weeks antenatal	50	No anti-D	A Rh D pos.	Negative	Nil	Yes
8	IUD 18 h antenatal	200	No anti-D	O Rh D pos.	Negative	Nil	Yes
2	IUD 4 days antenatal	400	Anti-C+D, anti-D enzyme reacting, anti-C titre 2	A Rh D pos.	Weakly positive	Nil, cord Hb 14·7 g/dl	Yes
9	Normal delivery, evidence of chronic feto-maternal haemorrhage, cord Hb 5·0 g/dl, reticulocytes present in cord blood	100	Anti-C+D, maximum 14·5 i.u./ml	A B Rh D pos.	Strongly positive	Clinical anaemia and jaundice, cord Hb 7·7 g/dl, cord bilirubin 132 mol/l	Yes after two exchange transfusions

lpf, Low power field; IUD, intrauterine death.

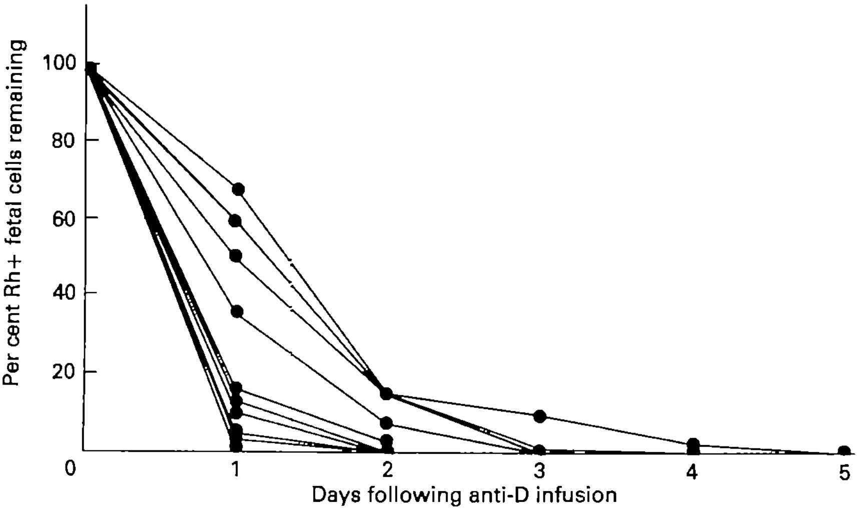

Fig. 1. Clearance rate of Rh D+ fetal cells following infusion of fresh plasma containing anti-D. The range of anti-D:fetal cell ratios was calculated to be 52·5–283 i.u./ml fetal cells (mean 129 i.u./ml).

unable to discover any recent reports about the likely outcome of management. In the early years of Rh immunoglobulin prophylaxis, failure to prevent sensitization in such patients was due to the use of inadequate doses of anti-D (Dudok de Wit & Borst-Eilers 1968; Hughes-Jones & Mollison 1968). Subsequently, management has largely been based on the experience of treating Rh-negative subjects transfused with Rh-positive blood (Keith *et al.* 1970; Pollack *et al.* 1971; Bowman *et al.* 1972; Mollison 1983), it being established that prevention of sensitization was possible if a sufficiently large dose of anti-D was given.

The 14 episodes reported in this series occurred over a period during which there were approximately 18 000 Rh-negative pregnancies, giving an overall incidence of 1 per 1300, similar to the incidence of 1 per 1000 described previously by Mollison (1983). Some of the estimated volumes of fetal blood loss in our series are unexpectedly high, bearing in mind the combined fetal and placental blood volume. This almost certainly reflects the wide margins of error inherent in the counting technique and indicates that the estimate should only be taken to be an approximate guide.

We did not use the usual intramuscular preparation of purified anti-D immunoglobulin because on the first occasion a sufficiently large dose was not available so instead we used fresh frozen plasma containing anti-D. Later we considered that this material, which could be administered intravenously, might have some other advantages, and Clarke (1969) had used this method successfully. Infusion of fresh plasma avoids the large and painful intramuscular injection with the required number of the 2500 i.u. vials available in the UK. In addition, intravenously administered anti-D rapidly achieves the maximum level of antibody which may be particularly important where the timing of haemorrhage is uncertain and fetal cells may have been present in the maternal circulation for some days. After intramuscular injection the maximum plasma level, only corresponding to 40% of the initial dose, is reached after 2–4 days (Mollison 1983). Delay in removing Rh D-positive cells is known to increase the likelihood of sensitization. Fresh frozen plasma does carry the potential for transmission of viral hepatitis and AIDS but the observed incidence of post-transfusion hepatitis B and non-A non-B hepatitis appears to be exceptionally low in the UK and therefore certainly at the beginning of this study these risks were not considered to be significant contraindications. All the fresh frozen plasma used was from donors participating in a regular anti-D plasmapheresis programme and was fully monitored and screened according to current guidelines (DHSS 1981, 1984*a*, *b*, 1985).

The dose of anti-D administered varied from 52·5 to 283 i.u./ml fetal cells (mean 129 i.u./ml). The recommended dose is 125 i.u./ml fetal cells using intramuscular preparations of immunoglobulin (WHO 1971); with the intravenous route a smaller dose of 60 i.u./ml fetal cells is recommended using a commercial preparation of anti-D immunoglobulin (WHO 1971). The rates of cell clearance did not appear to correspond with the dose of anti-D administered. This may have been due to variable factors including maternal plasma volume, fetal genotype, and anti-D immunoglobulin potency. Various sources advocate the use of divided doses of intravenous immunoglobulin to avoid the predicted haemolytic reaction arising from rapid cell destruction and a possible hypersensitivity reaction to the preparation used (Mollison 1983; Bowman 1982). In this series the plasma was infused slowly to allow close observation of the patient's condition. Most infusions were completed uneventfully but one patient did suffer a severe hypersensitivity reaction. This complication is a known risk of the use of fresh frozen plasma.

Conclusive evidence that sensitization has not occurred can only be obtained by following the progress of a subsequent pregnancy with an Rh-positive fetus. Four women achieved a successful second pregnancy; sensitization had in fact occurred in two of the four. In one this was not of sufficient severity to affect the clinical outcome. In the other, the baby survived despite being affected by haemolytic disease of the newborn. Interestingly, in this case, the presence of reticulocytes in the cord blood of the first baby suggested the occurrence of a chronic bleed. In all the remaining women anti-D had become undetectable by 9 months and this appears encouraging when the prospects for a future pregnancy are considered. How much this reflects the beneficial effect of the passively administered anti-D is uncertain. The risk of anti-D immunization following infusion of Rh D incompatible cells appears to range from less than 50% (Mollison 1983) during anti-D immunization programmes involving relatively small volumes of cells to over 80% when infusions of 200 ml red cells are given (Pollack *et al.* 1971). Bearing in mind the range of feto-maternal haemorrhages encountered in this series (50–400 ml) a proportion of the women would have been expected to show clear evidence of active anti-D formation.

In three women the feto-maternal haemorrhage was associated with intrauterine death and in these the timing of haemorrhage was uncertain. Certainly, in two, fetal cells were likely to have been present in the maternal circulation longer than the 72-h interval normally advised as a maximum during routine postnatal therapy. It is of interest that these women had a successful subsequent pregnancy. It is known that massive feto-maternal haemorrhage may be the cause of unexplained stillbirth (Whitfield *et al.* 1986). This study would suggest that where such a woman is Rh negative the presence of fetal cells in her circulation should be tested for and quantified in order to initiate early prophylactic treatment.

From this small series it appears that anti-D therapy can reasonably be expected to produce a successful outcome after massive feto-maternal haemorrhage, even on occasions when the haemorrhage has been prolonged. It is not suggested that fresh frozen plasma necessarily be used as the source of anti-D in this situation but intravenous anti-D does appear to have some advantage over the intramuscular preparation. Some attention may be necessary to the provision of large doses of anti-D in a convenient and comfortable form which ensure reliable recovery in the maternal circulation and an effective rate of fetal cell clearance.

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Surfactant stimulates prostaglandin E production in human amnion

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Summary. Discs of human amnion prepared from nine women delivered by elective caesarean section at term were incubated with and without purified human amniotic fluid surfactant (9 μg lipid P/ml), and the output of prostaglandin E (PGE) was estimated by radioimmunoassay. Surfactant stimulated the release of PGE from 3·8 (SD 2·9) to 5·4 (SD 2·5) pmol/mg dry weight/3 h ($P<0{\cdot}01$). Arachidonic acid accounted for 2% of the fatty acids of surfactant lecithin. These results show that surfactant has a stimulatory effect on amniotic PGE production and that it is an important source of arachidonic acid in amniotic fluid.

The factors responsible for the onset and maintenance of human labour remain unknown, but prostaglandins (PG) are likely to be involved. During pregnancy PG can stimulate myometrial contractility both *in vivo* and *in vitro* (Wiqvist *et al.* 1983) and PG synthesis inhibitors have been used successfully to inhibit uterine contractions in preterm labour. The onset and progress of labour is accompanied by increased intrauterine PG production, as reflected by PGE and PGF levels in amniotic fluid and levels of PG metabolites in the fetal and maternal circulation (Keirse 1979). Moreover, specific PGE receptors have been demonstrated in pregnant myometrium (Schillinger & Prior 1976).

The fetal membranes, especially the amnion, are an important intrauterine source of PGE, and it has been proposed that an activation of PG production in this tissue is the stimulus for the spontaneous onset of labour (Casey & MacDonald 1986). Since the amnion is an avascular membrane it has been proposed that its cells receive their supply of the PG precursor arachidonic acid from amniotic fluid (Okita *et al.* 1983), although the source of arachidonic acid in amniotic fluid has not been identified. In this paper we have investigated the possibility that surfactant, which is secreted by the fetus into the amniotic fluid, is an important source of arachidonic acid, and have studied the effect of purified surfactant on the rate of PGE production by amnion *in vitro*.

Materials and methods

Fetal membranes were obtained from nine women delivered by elective caesarean section at term before the onset of labour. The operations were indicated for previous section or breech presentation and were performed under epidural or general anaesthesia. Amniotic fluid was collected at artificial rupture of the membranes in women in spontaneous labour at term.

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Specimens from 3–10 women were pooled and processed as described later. All women were delivered of healthy singleton infants. Mid-trimester samples of amniotic fluid were obtained at amniocentesis for prenatal diagnosis. The tissues were collected in accordance with the requirements of the Central Oxfordshire research ethics committee.

Discs of amnion were punched with a 17-mm cork borer and placed in pseudoamniotic fluid (PAF, Schwartz *et al.* 1977) bubbled with 95% O_2–5% CO_2. The composition of PAF (in mmol/l) was: NaCl 220, $NaHCO_3$ 33, KCl 8, $CaCl_2$ 8, $MgCl_2$ 2·8, Na_2HPO_4 0·06, urea 6·2, glucose 2, HEPES 25, and fatty acid free human albumin (fraction V, Sigma Chemical Co. Ltd) 2 g/l. The discs were pre-incubated in PAF for 1 h at 37°C and the medium was discarded. The discs were then incubated in fresh PAF (1 disc/2 ml) alone or containing surfactant or other test substances. Incubations were made in quadruplicate; controls consisted of medium plus or minus test substances but without tissue. The viability of the discs was assessed by Trypan blue exclusion and by lactate production rates (López Bernal *et al.* 1987). After incubation the medium was acidified and extracted with ethyl acetate and the dry weight of the tissue determined. PGE was estimated by radioimmunoassay (López Bernal *et al.* 1987). The mean (SD) coefficient of variation of quadruplicate estimations was 16 (5)% (n = 20). In all experiments, PGE blanks in the controls were less than 5% of the corresponding PGE levels in the presence of tissue; therefore neither surfactant nor any of the test substances interfered significantly in the assay.

Human amniotic fluid surfactant was purified by centrifugation on sucrose gradients (Phizackerley *et al.* 1979). Surfactant lipids were extracted, purified by silicic acid column chromatography and separated by two-dimensional TLC (Poorthuis *et al.* 1976). Surfactant lecithin was hydrolysed and the fatty acids were extracted and measured by gas chromatography (Moore *et al.* 1975). Arachidonic acid was obtained from Cayman Chemical; calcium ionophore (A 23187) calcium salt, indomethacin

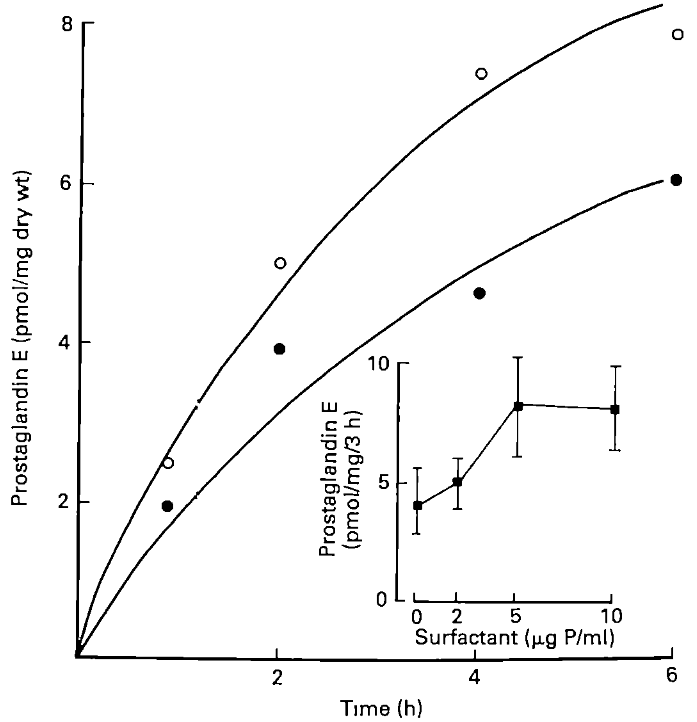

Fig. 1. Time course of PGE production by amniotic discs incubated in pseudoamniotic fluid alone or with added surfactant (9 μg lipid P/ml) as described in the text. The inset represents a dose response experiment Results are means (bars = SD) of incubations carried out in quadruplicate. ○, Surfactant; ●, control.

and phorbol 12-myristate 13-acetate from Sigma Chemical Co. Ltd; EGTA [1,2-di (2-aminoethoxy) ethane-NNN′N′-tetraacetic acid] from BDH Ltd and ritodrine HCl from Duphar.

Statistical differences were assessed by the Wilcoxon and Mann–Whitney tests.

Results

The production rate of PGE by amniotic discs was relatively constant for about 5 h (Fig. 1) and incubations were normally stopped at 3 h. To validate the system the effect of several stimulants and inhibitors of PG production was studied. A significant increase in PGE output resulted from the addition of arachidonic acid, ritodrine, calcium ionophore and phorbol ester. By contrast, EGTA (a calcium chelating agent) and indomethacin had strong inhibitory effects. The response to arachidonic acid was dose dependent (Fig. 2).

In the presence of surfactant the PGE production rate was increased and this was dose dependent (Fig. 1). In nine different patients the mean basal PGE output (pmol/mg dry weight/3 h) was 3·8 (SD 2·9) and in the presence of surfactant it increased to 5·4 (2·5) ($P<0{\cdot}01$); individual values are plotted in Fig. 3.

The phospholipid distribution of three different surfactant preparations at term was: phosphatidylcholine 80·6 (SD 1·9)%, phosphatidylethanolamine 2·5 (0·4%), phosphatidylglycerol 5·7 (0·6)%, unidentified 11·2 (2·2)%. The fatty acid content of surfactant lecithin was: palmitic 86·6 (3·0)%, stearic 2·2

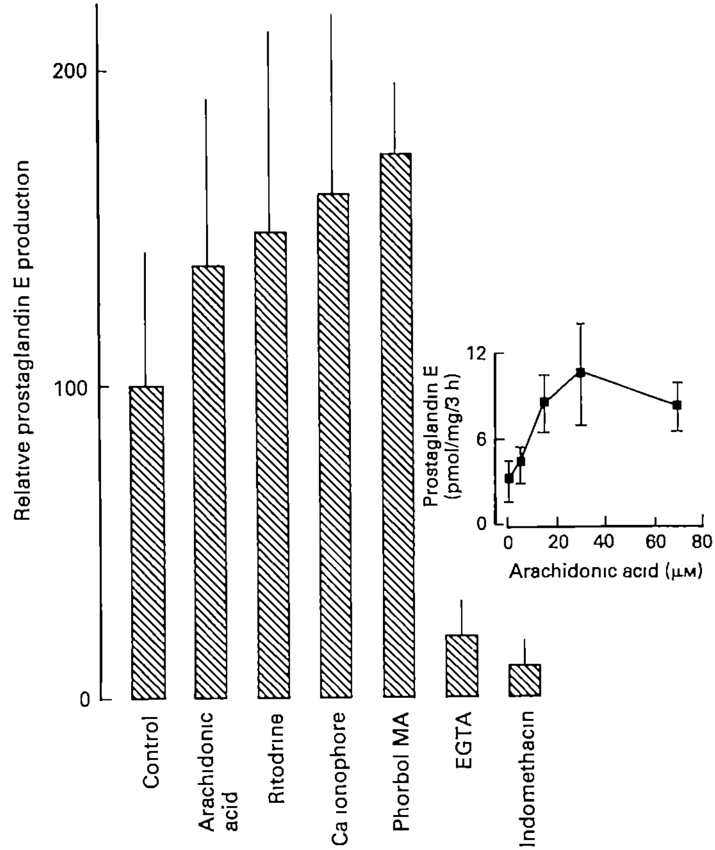

Fig. 2. Effect of several compounds on PGE production by amniotic discs. The histograms represent the mean of 4–5 different experiments and the bars are SD. The following concentrations were used: arachidonic acid 5 µM, ritodrine 10 µg/ml, calcium ionophore 10 mM, phorbol myristate 10 mM, EGTA 1 mM, indomethacin 10 µM. The inset represents a dose response experiment. Results are means (bars = SD) of incubations carried out in quadruplicate

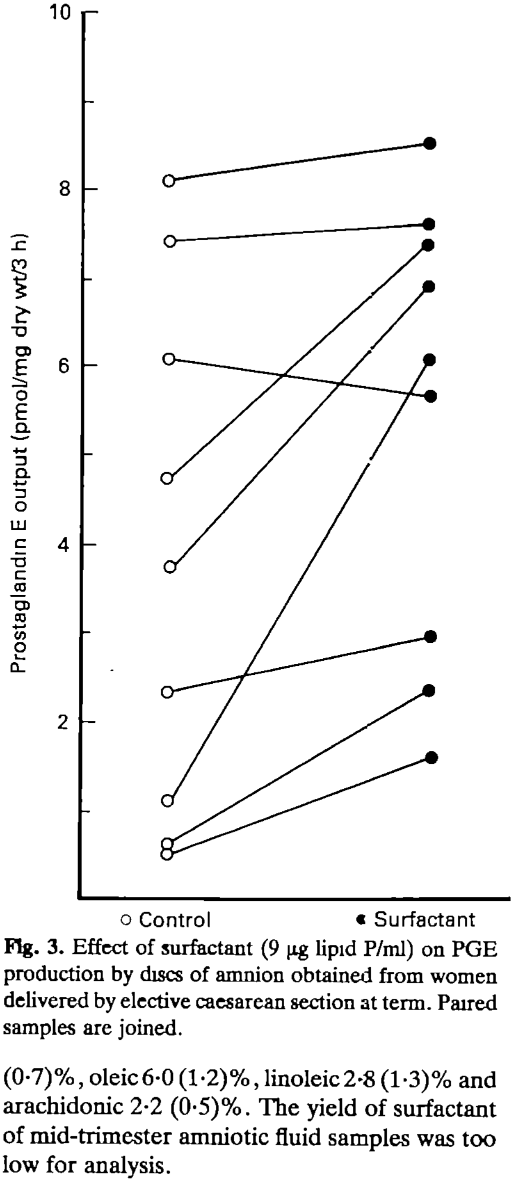

Fig. 3. Effect of surfactant (9 μg lipid P/ml) on PGE production by discs of amnion obtained from women delivered by elective caesarean section at term. Paired samples are joined.

(0·7)%, oleic 6·0 (1·2)%, linoleic 2·8 (1·3)% and arachidonic 2·2 (0·5)%. The yield of surfactant of mid-trimester amniotic fluid samples was too low for analysis.

Discussion

These results show for the first time that purified human amniotic fluid surfactant stimulates PGE production by term amniotic discs. Amniotic discs are easy to prepare and remain metabolically active for several hours (López Bernal *et al.* 1987). Under our incubation conditions, PGE output was stimulated by arachidonic acid, by a beta-mimetic and by a phorbol ester (Bleasdale & Johnston 1985) and was dependent on extracellular calcium (Olson *et al.* 1983), which suggests that this is a good preparation for the study of factors that modulate PG production.

The mechanism by which surfactant increases PGE production is not known. It is believed that availability of free arachidonic acid is the rate-limiting factor for PGE synthesis in amnion (Bleasdale & Johnston 1985). In-vitro studies suggest that amnion has a high turnover rate for arachidonic acid (Schwartz *et al.* 1977) and it is possible that surfactant phospholipids are taken up by this tissue and metabolized to release free fatty acids including arachidonic acid. Phospholipase A_2, phospholipase C, di- and monoacylglycerol lipase activities are present in amnion (Bleasdale & Johnston 1985). Alternatively, surfactant may activate lysosomal enzymes in amniotic cells (Sbarra *et al.* 1983), including phospholipases, and this would result in increased PGE production from released endogenous arachidonic acid. The observed variation in response between patients may be due to differences in the availability of endogenous arachidonic acid. It is not known whether the amnion contains specific receptors for surfactant, but the presence of surface active materials in the amniotic epithelium has been demonstrated by hydrophobicity measurements (Hills & Cotton 1986).

Our results show that arachidonic acid accounts for about 2% of fatty acids of surfactant lecithin. Previous workers (Das *et al.* 1975) have estimated that 2% of fatty acids of total lipids in amniotic fluid is arachidonic acid, and the proportion in amniotic fluid lecithin is 1–10%. Our findings, therefore, demonstrate that surfactant is a major source of arachidonic acid in amniotic fluid.

The amnion is believed to play a crucial role in the initiation of human parturition through the generation of PGE. Surfactant represents a rich source of arachidonic acid which can be utilized for PG synthesis by the fetal membranes; the dose response curves for surfactant and arachidonic acid are similar, although our data do not exclude the possibility that surfactant stimulates endogenous PG production. Experiments are now in progress to characterize the effect of lipid and protein fractions from surfactant.

While surfactant can stimulate amniotic PGE production, the fact that its composition remains stable during the final days of gestation (Moore *et al.* 1975; Kulovich *et al.* 1979) makes it unlikely that surfactant is directly responsible for the acute increase in intrauterine PG production associated with the spontaneous onset of human labour. Nevertheless, surfactant may well be involved in the mechanism, for it provides a link between the process of maturation of the fetus in preparation for birth and the potentially labour-initiating activation of PGE synthesis in the amniotic epithelium.

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Fetal heart rate monitoring by telephone. I. Development of an integrated system in Cardiff

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Summary. We report the development of a practical dedicated system for domiciliary fetal monitoring integrated in a scheme for its rational application. From experience of 1120 domiciliary recordings in 74 women (64 with high-risk pregnancies), we suggest that domiciliary monitoring applied within a structured clinical context should be as safe as monitoring in hospital.

Unstressed antenatal fetal heart rate (FHR) monitoring remains widely used in the immediate assessment of fetal welfare but has not fully realized its potential (Grant & Mohide 1982). The value of unstressed tests might be altered by the frequency of testing, and by alteration of other factors, such as primary screening, selection, and consistency in interpretation of recordings. The need for frequent monitoring may require lengthy admission to hospital or frequent attendances with accompanying counterproductive stresses for the woman (Wilson-Barnett 1976), the fetus (Newton 1985), and the family (Merkatz 1978).

We report the development of a dedicated system suitable for domiciliary fetal monitoring by midwives, or by women themselves, integrated in a scheme for its rational application.

Patients and methods

Antepartum FHR monitoring by telephone

The feasibility of domiciliary antenatal FHR recording using the public telephone network was described by Dalton *et al.* (1983). Audible fetal heart sounds were relayed in real-time from patients' homes, with demodulation and secondary computer processing in the hospital. In 1983 we began the development in Cardiff of a digital system for the rapid transmission of compressed data (Gough & Dawson 1986). Beat-to-beat intervals were measured and stored in a home recording unit, then later they were transmitted by telephone to the hospital computer, taking about 4 min for a 20-min recording. Neither method made the best use of available technology, and neither allowed for the transmission of fetal movement events or uterine activity.

Prototype domiciliary fetal monitor

In a further, microprocessor-based version (Gough *et al.* 1986), FHR data were collected and reduced in real-time by the home recording unit, now called a domiciliary fetal monitor (DFM). The DFM comprises a small portable case containing a conventional widebeam ultrasound transducer, two controls, and rubber cups to accept the telephone handset when the 30-min recording is to be transmitted. During recording correct positioning of the transducer is facilitated by an audible 'beep' synchronous with the fetal heart sounds. Just before transmission, details of identity, date and time are entered on the hospital BBC microcomputer screen.

The reduced data are transmitted to the hospital using normal telephone lines in less than

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40 s, providing a visual FHR trace for immediate assessment. Transmission is controlled automatically by the central computer and is protected against noisy lines or interruption. The DFM audibly indicates the end of a successful transmission. The central computer also displays, prints, and stores the recordings. This system overcame the major drawbacks to close monitoring without the need for hospital admission, and also has the potential for extra registration channels. It has been used in our introductory clinical trials reported here, and remained in use for the commencement of a randomized controlled study, to be reported soon.

The Huntleigh DFM system

In a co-operative venture, one of us (N.A.J.G.) has supported engineers at Huntleigh Technology PLC in developing the microprocessor-based system into the Huntleigh DFM system, which has now undergone independent clinical trials in four other centres, as reported by James *et al.* (1988).

Developments in the remote DFM (Fig. 1) have included operational software changes leading to improved signal quality and more reliable transmissions, a fetal event marker, a simple one-button control, and a more robust battery supply with mains by-pass. Each DFM transmits a unique identity code.

The central station (Fig. 2) has been upgraded to IBM compatibility and uses a more reliable hardwired modem. The DFM code number is printed on the record to assist in accurate identification when several DFMs are in use. In the production version there is capacity for extra clinical information to be recorded on screen and on paper (Fig. 3), whilst in a single modified version recently installed in Cardiff, recordings are also held in a database with a limited registration file.

Domiciliary surveillance

Integration of domiciliary monitoring in Cardiff. Whilst developing our DFM system we have also turned to setting up more specific patterns of antenatal care, resulting in the Cardiff Integrated Antenatal Care (CIAC) Scheme, described in more detail elsewhere (Middlemiss

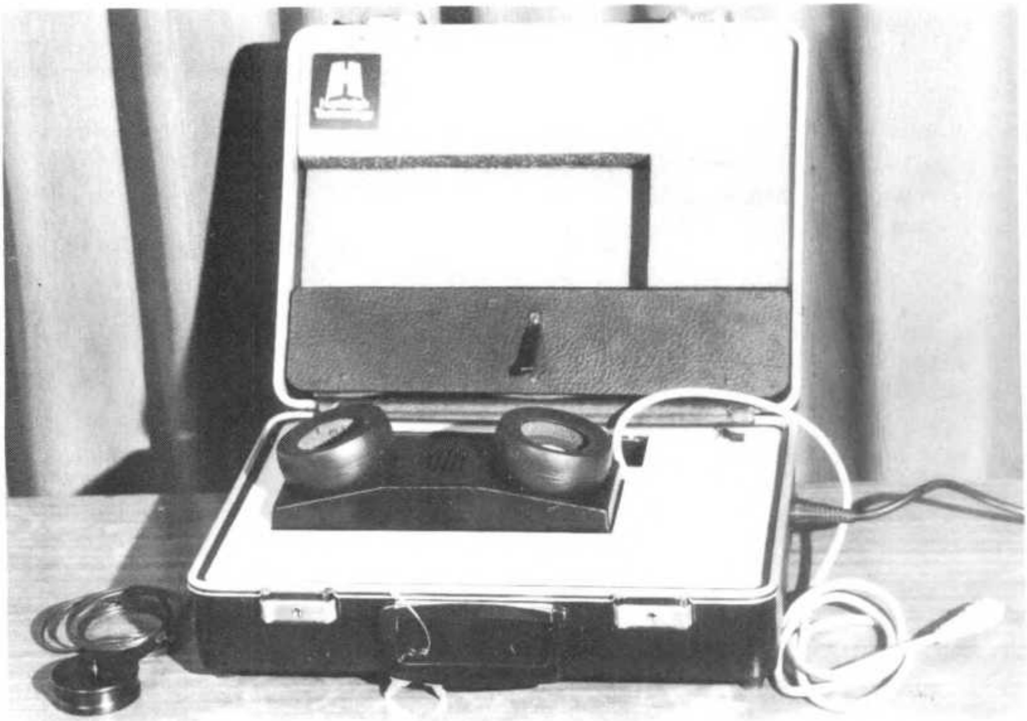


Fig. 1. Remote production model domiciliary fetal monitor (DFM). Briefcase-style container showing rubber cups for telephone, ultrasound transducer (left), fetal event marker (right), and simple touch-button control panel.

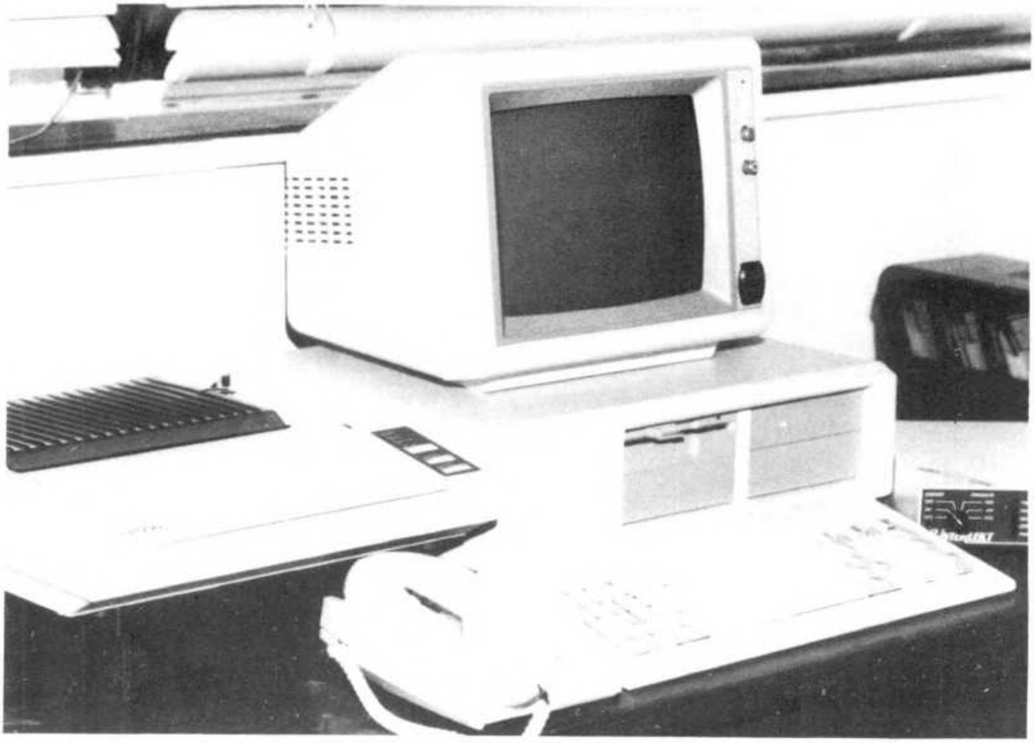


Fig. 2. Central station for commercial version comprising IBM-compatible personal computer and VDU, with dot-matrix printer (left) and hardwire modem (right of keyboard). The telephone is switched between voice and modem communication modes.

et al. 1987). The scheme aims to provide comprehensive domiciliary surveillance in selected women with pregnancies at increased risk. Advice and encouragement are provided in the women's homes by midwifery sisters, who communicate with the obstetrician directly and by telephone.

Selection for domiciliary surveillance. We recruited 10 healthy volunteers and 64 women with previously identified factors for increased fetal and maternal risk. Women were not considered suitable for domiciliary care if they were likely to require acute intervention. Selection was not influenced by their distance from the hospital, or by whether they were telephone subscribers.

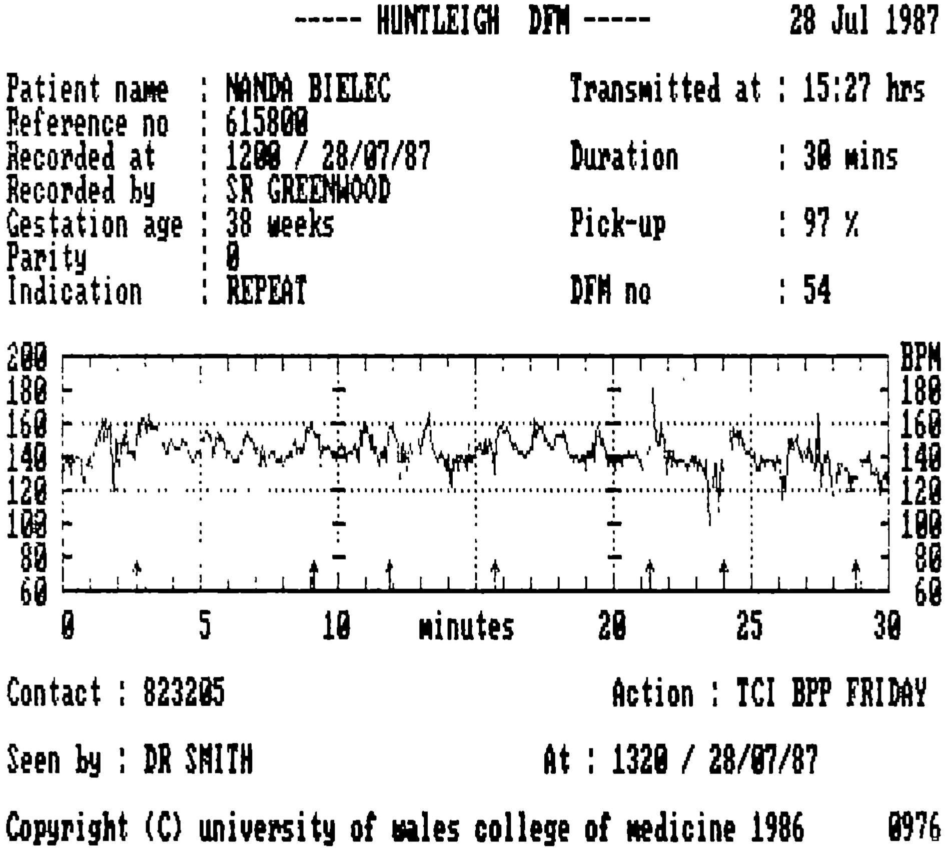
Monitoring technique. The DFMs are independent of a telephone during recording. The FHR recordings are made either by the midwife or the woman herself, and fetal movements are noted during the recording. The finished recording is transmitted by telephone to the hospital for early review by prior arrangement, with one of us manning the central station. Recordings were made mostly during the day, and the women usually made and telephoned their own recordings at weekends and on occasional weekdays. Rarely, when recordings were clinically dubious or of poor quality, either they were repeated or the woman was brought to the hospital, as appropriate.

Results

Transmissions

Up to November 1987 we have transmitted 1120 recordings in 74 women. Of these, 55% were made by midwives, and 45% by the women themselves. We have provided care in individual pregnancies from as early as 27 weeks, and for up to 109 days. Three women could not be trained to make their own recordings.

In a randomly selected series of 100 consecutive recordings the average delay between the completion of a recording and its receipt in the hospital was 32·9 min (SD 47·37 min). Delays of up to 2 h occurred infrequently, and



----- HUNTLEIGH DFM ----- 28 Jul 1987

Patient name : WANDA BIELEC
Reference no : 615800
Recorded at : 1200 / 28/07/87
Recorded by : SR GREENWOOD
Gestation age : 38 weeks
Parity : 0
Indication : REPEAT

Transmitted at : 15:27 hrs
Duration : 30 mins
Pick-up : 97 %
DFM no : 54

Contact : 823205

Action : TCI BPP FRIDAY

Seen by : DR SMITH

At : 1320 / 28/07/87

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Fig. 3. Hard copy paper printout of recording and other information immediately following transmission. Produced by 'dumping' screen display using dot-matrix printer (production version). Fetal events are shown by arrows.

only at weekends. Time on the telephone during this study was rarely more than 5 min.

Eighteen women (24%) were not telephone subscribers and then we were often able to use a neighbouring telephone. For non-subscribers, since August 1986 we have used a portable cellphone to transmit 77 recordings.

On 11 occasions the obstetrician reviewed the recordings at home using his own microcomputer.

Problems with transmissions

In addition to the 1120 recordings transmitted, 11 were made but lost because of failed transmission. Also during the developmental phase there were less serious difficulties, sometimes combined: on 34 occasions because of failed arrangements between personnel or patients, on 55 occasions because of poor modem operation, and on 47 occasions for other multiple reasons, mostly due to equipment failure. All were eventually dealt with by various means, mainly by repeating the attempt at transmission, or occasionally by carrying the units into hospital for direct hardwire transmission. We have investigated and resolved these technical problems during development of the Huntleigh DFM system.

Acceptance times

We have used the positive concept of 'acceptance time' to measure signal loss. This is the proportion of recording time for which reduced heart rate data (Dawes *et al.* 1981) are available. Acceptance time is complementary to the method first described by Dawes *et al.* (1981), whose equivalent data showed that the acceptance time for apparently good conventional ultrasound recordings would average 79%. Acceptance times for 493 recordings in 40

Table 1. Frequency distribution of acceptance times for 493 serial recordings during 40 pregnancies

%	*n*
1–10	0
11–20	3
21–30	8
31–40	16
41–50	46
51–60	65
61–70	103
71–80	123
81–90	99
91–100	30

These values incorporate changes from improvements during development.

pregnancies are given in Table 1. More comprehensive information about acceptance times and the effect of gestational ages is given by James *et al.* (1988).

Interpretation

Of the 1120 recordings transmitted, 13 (1%) were considered to be uninterpretable. Sixteen were equivocal, and 25 were recognized as abnormal, that is, with absent high variation (5), absent reactive accelerations (4), decelerations (12), tachycardia with or without decelerations (2), and sinusoidal rhythm (2).

Description of outcome

The DFM system contributed to a confident decision to intervene urgently in three pregnancies, all with successful outcome. In each of them daily monitoring revealed the development of low variation in heart rate with decelerations. The findings were confirmed by conventional recordings before delivery.

The remaining women all avoided hospital admission or frequent clinic attendance for varying periods, and were reassured by the care they received. Fourteen received intervention for reasons other than FHR abnormalities. In no case did domiciliary monitoring fail to reveal an adverse condition.

Compliance in our high-risk women to date has been good with all but one. There were no clinic defaulters and no self-discharges from hospital.

Discussion

We have shown that domiciliary unstressed FHR testing can be carried out safely and efficiently in several women at a time. The method has already demonstrated its capability of alerting the obstetric services to potential problems in high-risk pregnancies, even for non-telephone subscribers. Because recordings are rapidly transmitted and reviewed the fetal monitoring schedule is economical of time and money for both patients and attendants. This design therefore represents a considerable advantage over a system operating in real-time through an open telephone line (Dalton *et al.* 1983; Dalton & Currie 1986). Additionally, the cellular telephone has proved a more flexible and cheaper answer for non-subscribers than new permanent telephone installation (Currie & Dalton 1986).

The lack of a fetal event marker we occasionally found to be frustrating as the absence of accelerations in FHR might simply have been due to temporary fetal inactivity. This prompted our inclusion of an event channel in the Huntleigh DFM.

Our experience has been that with brief training most women can confidently make their own recordings unsupervised, in broad agreement with the Cambridge group (Currie & Dalton 1986). However, it is vital that women who have been identified at risk should not receive isolated fetal monitoring at home, and out of a clinical context (Grant & Mohide 1982). With the midwife visiting the patient, rather than acting as a monitoring technician, general compliance is excellent, and we are already finding that we can reduce the number of clinic visits and hospital admissions to great benefit.

Digital domiciliary fetal monitoring may have considerable application in complementing outpatient antenatal care. Several other applications for DFM from other centres are discussed by James *et al.* (1988), together with jointly formulated recommendations for other centres taking up this useful facility. A detailed checklist is available from the Cardiff Group.

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Fetal heart rate monitoring by telephone. II. Clinical experience in four centres with a commercially produced system

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Summary. A commercially produced domiciliary fetal monitoring (DFM) system was assessed in four centres in the UK (Bristol, Glasgow, London and Nottingham) chosen to allow for comprehensive assessment in various settings in many different women. Overall, 825 recordings were made from 368 women (2·24 per woman). There were 56 unsuccessful attempts at transmission (6·8%), most were due to problems with telephone equipment. The system worked most efficiently when a dedicated direct line was used. The data transmission time varied between 40 and 60 s. The median telephone time (including data transmission and conversation) with a dedicated direct line was 7 min. Mean acceptance times from the four centres were between 70 and 80%. All recordings with acceptance times of 40% or more were interpretable. Ten recordings were abnormal. The women and midwives were equally proficient at using the DFM system. The DFM system represents an important addition to current methods of fetal assessment. Specific guidelines are outlined.

Unstressed antenatal fetal heart rate (FHR) monitoring remains arguably the most commonly employed objective method of assessing fetal health (Montan *et al.* 1985). Dawson *et al.* (1988) discussed the disadvantages of 'bringing the patient to the machine' either as an outpatient with frequent hospital attendances or as an in-patient, and they described the technical

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development of a domiciliary fetal monitoring system. Clinical and technical assessment of the first production model is now described as assessed over 3 months in four centres in the UK.

Patients and methods

Production model

The production model of the domiciliary fetal monitoring system, Huntleigh DFM (Huntleigh Technology PLC, Healthcare Division, Cardiff), described by Dawson *et al.* (1988) consists of three parts:

(i) Remote telephone units (domiciliary fetal monitors) (DFM) which may be used by the woman or her community midwife either in the home or in the local health centre. The DFM records, compresses and stores a 30-min fetal heart waveform together with fetal movements recorded simultaneously by the woman using a hand-held event marker. The DFM has an integral acoustic coupler which enables the compressed data to be transmitted along telephone lines and it can be battery or mains operated. For the field trials five DFMs were used at each of the four trial centres.

(ii) A hospital base station comprising a PC type microcomputer system, printer and direct line modem.

(iii) Standard British Telecom telephone lines.

The DFM system works in the following way. When a call is received at the hospital base station from a patient or midwife with a FHR record stored on a DFM, the person at the hospital receiving the call first takes certain basic clinical information from the caller and enters it into the computer. The base station operator then instructs the sender to place her handset into the acoustic coupler in the DFM and to switch the DFM to 'send' mode. The base station operator simultaneously switches the base station modem to 'acquire/receive' mode. Thereafter the microcomputer 'instructs' the DFM to release its compressed, stored data along the telephone line. Once all the stored data have been transmitted, the base station automatically (via the DFM) signals the sender to pick up her handset. The microcomputer 'unscrambles' the signal and the FHR trace with event marks is displayed on the VDU. The receiver can interpret the trace and advise the sender before terminating the call. Subsequently, hard copies of the trace are printed.

Organization of the field trials

Clinical and technical assessment of the DFM system was undertaken in four teaching hospital centres in the UK—Southmead Hospital, Bristol (5758 deliveries in 1986); Glasgow Royal Maternity Hospital (4301 deliveries in 1986); the City Hospital, Nottingham (5620 deliveries in 1986) and the Royal Free Hospital, London (2035 deliveries in 1986). The rate of unstressed antenatal FHR monitoring varies between 10 and 15% for the four hospitals. The organization of the use of the DFM system varied between the four centres.

Southmead Hospital, Bristol. The five DFMs were mainly used by community midwives at five health centres around the periphery of the city (Lawrence Weston, Cossham, Chipping Sodbury, Clevedon and Thornbury). Most traces were recorded in the health centre itself with a few being recorded in the patient's home. In most cases DFM monitoring was performed no more than twice weekly. However, towards the end of the field trial, three women used a DFM on a daily basis in their own home. The calls were made through the hospital switchboard to a dedicated extension on the hospital delivery suite where the base station was housed. Thus staff experienced at FHR trace interpretation were available to accept calls 24 h each day and 7 days each week.

Glasgow Royal Maternity Hospital. The five DFMs were used by midwives. The units were distributed as follows: two were used in Glasgow both in the hospital's fetal assessment unit and in the patients' homes; one in Cresswell Maternity Hospital, Dumfries; one in Lewis Hospital, Stornaway; and one in Daliburgh Hospital, Uist. The calls were made to the hospital base station situated in the fetal assessment unit. For the first half of the field trials an extension from a mini-switchboard was used. This was not a dedicated but a shared line and caused significant delays in the handling of transmissions (see later under *Telephone times*). Thus for the second half of the trials a separate direct line was installed to solve these problems. The hospital base station was manned 8 h each day and 5 days each week.

Royal Free Hospital, London. The five DFMs were issued to individual women after an initial training at the fetal assessment unit by the midwife in charge. The women undertook daily traces in their own homes. They telephoned the traces through to the fetal assessment unit where the base station was located. A separate direct line was used for the calls. The base station was manned 4 h each day, Monday to Friday inclusive.

City Hospital, Nottingham. The five DFMs were used by midwives in the patients' homes. The recordings were made twice weekly in most cases. The base station was located on the antenatal ward and was manned 8 h each day and 5 days each week. A separate direct line was used for the base station.

Protocol for use of DFM system, interpretation and management

The DFM system was used in the four centres during the field trials in accordance with an agreed protocol. Once a woman had been identified as having an indication for FHR monitoring, she would have a 30-min FHR recording made twice weekly using the DFM system. More frequent FHR testing and other investigations were made at the discretion of the consultant in charge of the case.

A 'normal' trace was identified as having a baseline of 120–160 beats/min, two accelerations of at least 15 beats/min and lasting for at least 15 s and no decelerations. When a trace was thus interpreted as 'normal' the patient would have a repeat recording made within 3–4 days.

An 'abnormal' trace was identified as either having a baseline <120 beats/min or >160 beats/min, or less than two accelerations or the presence of decelerations. When a trace was thus interpreted as 'abnormal' the woman was to attend the hospital immediately for a conventional cardiotocograph (CTG) and/or biophysical profile.

If the trace was unsatisfactory for technical reasons the woman or midwife making the recording was asked to repeat the procedure later the same day. If the technical problems persisted then the woman attended the hospital for a conventional CTG and/or biophysical profile.

A DFM recording had to be transmitted and interpreted as soon as possible and always within 3 h of starting the recording.

Results

Indications for FHR monitoring

A total of 368 women had FHR monitoring using the DFM system on one or more occasions. The two main indications were reduced fetal movements (20·8%) and hypertension (14·4%). Other indications included previous stillbirth, post-term pregnancy, poor maternal weight gain, small-for-dates pregnancy and antepartum haemorrhage. A total of 75 normal pregnancies had FHR recordings made, either to teach midwives how to use the system or to test the equipment. Many of these recordings were undertaken using the hospitals' internal telephone lines for convenience.

Transmissions for FHR recordings

A total of 825 recordings were made from 368 women (2·24 recordings per woman) and 769 (93·2%) were successfully transmitted along the telephone lines to the base station. The details of the 56 unsuccessful attempts at transmission of the recordings from the DFM (6·8%) are described below.

Problems with the telephone lines accounted for most of the unsuccessful transmissions (43 of 56, 76·8%; or 5·2% of all recordings). Forty of these (93%) were in the Glasgow centre and represented a combination of the problems associated with the mini-switchboard mentioned above and adverse weather conditions affecting transmissions from the Western Isles to the mainland. Two of the remaining three unsuccessful transmissions were due to interruption of calls by the hospital switchboard network at Southmead Hospital. This was solved by making the extension a dedicated inviolate line.

Nine of the 56 unsuccessful attempts at transmission were due to human error (16·1%; 1·1% of all recordings). These included failure to pick up the handset once data transmission had been completed, incorrect operation of both remote and base units and inadvertent wiping out of the recording before it had been transmitted. Future production models will incorporate a modification to prevent this latter problem. Four of the unsuccessful attempts at transmission were due to equipment problems (7·1%; 0·5% of all recordings). All four were due to the battery on the DFM not being recharged which resulted in it being possible to record the fetal heart rate but not possible to transmit the recording. This problem does not arise when either the battery is charged or the DFM is used solely on mains power.

Telephone times

Telephone times, which include the times for data transmission and preceding and subsequent conversation, were influenced by the type of telephone connection at the base station site. In the centres with direct lines (London and Nottingham) the median time for the total telephone call was 7 min (range 5–15 min). Where the hospital switchboard extension was used (Bristol and Glasgow) the median time for the total telephone call was 12 min (range 5–25 min).

Alterations were made to the telephone systems used in Bristol and Glasgow during the field trials. In Bristol this comprised converting the switchboard extension to a dedicated inviolate line. In Glasgow this comprised the installation of a separate telephone line. In both centres these measures resulted in median total telephone call times (6 and 7 min respectively) being comparable to those in the other two centres. The time taken for data transmission was between 40 and 60 s.

Acceptance/pick-up

The DFM system calculates and displays an index of trace acceptance or pick-up, which is derived from the digital signal generated by the fetal heart rate record. This is equivalent to the reciprocal of loss of contact. The mean acceptance/pick-up of all recordings from the four centres were 71·2, 77·5, 75·0 and 79·5% respectively from Bristol, Glasgow, London and Nottingham.

Table 1. Effect of gestational age on acceptance/pick-up

Gestation (weeks)	Number of transmissions	Mean acceptance/ pick-up (%)
24–27	15	46·7
28–31	4	46·7
32–35	41	68·4
36–39	53	74·6
40–42	10	74·7
Overall	123	68·2

The factors which influenced acceptance/pick-up included gestational age/fetal size (Table 1), maternal obesity and hydramnios. The experience of the DFM user also influenced the acceptance/pick-up. For both patients and midwives there was a comparable learning curve for use of the DFM, both achieving similar optimum proficiency by their fifth attempt.

Interpretability

Three of the four field trial centres arbitrarily decided to classify the DFM trace as 'non-interpretable' if the acceptance/pick-up was less than 75%. Of the 825 recordings 38 (4·6%) were reported as non-interpretable by this definition. All 38 recordings in fact were interpretable and normal on subsequent review by the local consultant in charge of the DFM system.

It is our opinion that the DFM traces were interpretable when the acceptance/pick-up exceeded 40%. Only two traces were non-interpretable for this reason (0·24%). Both were from a pregnancy at less than 26 weeks gestation.

Eight recordings (0·97%) were reported as non-interpretable by the midwives or doctors receiving the call but were subsequently considered to be interpretable and normal on subsequent review by the local consultant in charge of the DFM system. In these eight recordings it appeared that the 'non-interpretable' report resulted from inexperience at reviewing DFM traces compared with conventional CTG traces.

Abnormal traces

The vast majority of traces were normal, 815 of 825 (98·8%) in 358 of the 368 women (97·3%). Ten recordings (1·2%) were abnormal; seven had decelerations and three were non-reactive with no accelerations.

Decelerations. Five of the seven women had repeat CTGs in hospital and as the abnormality persisted delivery was expedited. In four instances a healthy baby was born, the fifth was born by caesarean section at 27 weeks gestation and died at 7 days from the effects of extreme preterm delivery (27 weeks). In the other two women with decelerations, the CTG was repeated in hospital and was normal. In one, DFM recording, continued on an outpatient basis, remained normal and a healthy infant was born spontaneously at term. In the other no further fetal monitoring was undertaken (at the decision of the unit in charge of the patient) and 3 weeks later a well-grown, normally formed baby was stillborn at 39 weeks gestation.

Non-reactive. In two of the three women in this group CTGs repeated in hospital were normal. DFM review, continued on an outpatient basis, was normal, and both women gave birth to healthy babies after a normal labour and delivery. In the third woman CTG monitoring repeated in hospital was found to be abnormal (persistence of unreactive trace and the presence of decelerations) and delivery was effected resulting in a healthy baby.

Reactions from women and professional staff

The DFM system was generally favourably received both by the women and the professional staff. For the women the main benefits were that they did not have to travel to or stay in the hospital with associated family disruption. They also commented on the great reassurance they got from being able to hear the baby's heart beat.

From the professional staff's point of view the main advantages were the reduction in the numbers of women who required FHR monitoring in hospital. Even though with both conventional monitoring and the DFM system the midwives or obstetricians had to interpret the traces, they felt that this was less time-consuming with DFM than conventional CTGs since they did not have to 'set-up' the mother on a monitor. Community midwives found a 'normal' report from the hospital on a DFM trace extremely reassuring when they recognized a fetal risk factor.

The only adverse comments that were made by a few women were related to the cost of the telephone bills. There were two principal criticisms from professional staff. First, where the DFM recordings were undertaken by community midwives this did result in an increase in their workload. The second comment came from community midwives in Glasgow who were used to bringing their 'at-risk' pregnancies into hospital as day cases, performing their CTGs and interpreting them: they said they found the inability to see the FHR record in real-time and interpret it on the spot to be a disadvantage compared with the normal arrangement.

Discussion and recommendations

The commercially produced domiciliary fetal monitoring system represents a significant addition to present methods of fetal assessment. The field trials were intended to assess the value of the system rather than antenatal FHR monitoring itself.

The remote telephone unit (DFM) comes in a compact, robust and light (4·5 kg) attaché case which is easy to use. The hospital base unit uses a conventional IBM or compatible PC. Normal telephone lines can be used with no special adjustments. Undoubtedly, as advances are made in telecommunications, so the DFM system will adapt to become simpler in operation; for example, in time it will be possible to dispense with the acoustic coupler in the DFM and replace it with an integral modem. Other features will be incorporated, such as a contractions channel.

The cost of the system compares very favourably with conventional CTG monitors. For example, £20 000 will buy the hospital base unit and 15 remote units: the same sum will only purchase four conventional antenatal FHR monitors at best. Admittedly, the DFM system does not have the advantage of immediate interpretation but this is not mandatory for antepartum use provided transmission and interpretation are undertaken quickly.

The choice of the four field trial centres allowed the system to be compared comprehensively in a variety of settings (rural and urban, connurbation and remote isles), with a variety of users (women and midwives) and in a large number of women with a variety of different indications. Most of the unsuccessful transmissions were experienced in the Glasgow centre and were related to the mini-switchboard in use initially, and weather-related telecommunication problems. Such problems were not experienced elsewhere or indeed after the introduction of the direct line in Glasgow. The human errors resulting in unsuccessful transmission will be lessened with experience. The 'unsuccessful' transmissions due to equipment failures were due to failure to keep the battery well charged. The equipment in fact was very robust for the duration of the field trials and no inherent technical failure occurred in the manufactured equipment. In our opinion, the unsuccessful transmission rate could be kept below 2%.

The DFM system has average acceptance/pick-up times between 70 and 80% which are similar to those reported for conventional CTGs (Dawes *et al.* 1981). We believe that an acceptance/pick-up over 40% is normally sufficient for interpretation of a DFM recording and the only two that were not interpretable because of low acceptance/pick-up were in pregnancies less than 30 weeks. Experience in the interpretation of DFM traces is necessary since it takes time to adjust to the differences from the conventional CTG traces.

The women certainly appreciated the benefits of DFM, apart from the cost.

The DFM system reduces the workload for hospital staff (obstetric and midwifery) but where the remote units are used by community midwives it will increase their already heavy workload. The finding that women and midwives appear to be equally proficient at using the DFM is encouraging in this respect. It would seem logical that community midwives use DFM to assess fetal health on a single occasion; but where daily or alternate day monitoring is required women should be issued with their own system.

Whilst the DFM does not produce a real-time record, antepartum FHR traces recorded in hospital are not usually interpreted in real-time. Thus the DFM system in this regard is not that different from conventional FHR monitoring provided transmission and interpretation of the stored record is undertaken as soon as possible. We recommend that this should occur preferably immediately but always within 1 h of the start of the recording. Like all antepartum recordings of the FHR it is not an index of fetal health in itself. The FHR record should only be part of a comprehensive clinical, biochemical, biophysical and physiological assessment which should be undertaken when a fetal risk factor is identified in a pregnancy.

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Chorionic villus sampling in a high-risk population—4 years' experience

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Summary. Between August 1982 and July 1986 a total of 163 pregnancies in 136 patients with a high genetic risk have had prenatal diagnosis by chorionic villus sampling. Villi were not obtained in five pregnancies (two of which subsequently miscarried), and 11 fetal losses followed successful sampling (four in pregnancies in which the fetus was shown to be affected). The rate of fetal loss fell with increasing experience of the team. Three sets of twins were all sampled successfully.

Although the experience from China of fetal sexing using chorionic villi (Anshan 1975) was curtailed for ethical reasons, it demonstrated that 'blind' aspiration of villi was a comparatively safe approach to early fetal diagnosis. When it became apparent that DNA analysis of chorionic villi would permit early diagnosis in some couples at risk of a haemoglobinopathy (Williamson *et al.* 1981) we looked to the Chinese experience, which had gone largely unnoticed, in developing an ultrasound-guided technique for transcervical chorionic villus sampling (CVS), using a plastic catheter developed in collaboration with Portex Limited ('Trophocan', Portex Ltd, Hythe, England) (Ward *et al.* 1983). This was used in the first diagnostic series for haemoglobinopathies (Old *et al.* 1982) and subsequently with various modifications by many groups throughout the world (Jackson 1987). In the last 4 years CVS has been applied at our centre predominantly for couples with a high genetic risk, in most of whom prenatal diagnosis would formerly have been by fetoscopic blood sampling.

Patients and methods

Between August 1982 and July 1986, 136 women with a high genetic risk had prenatal diagnosis by CVS in 163 pregnancies.

Many of the women at risk of a haemoglobinopathy were already known to us because they had attended for prenatal diagnosis in earlier pregnancies. Other patients, referred with a variety of indications, had elected to have CVS rather than mid-trimester amniocentesis or fetoscopy. Because resources were limited, fetal karyotyping was possible only in those pregnancies where there was an additional indication such as advanced maternal age. All the couples were counselled and appraised of the uncertainties of CVS, particularly in relation to fetal loss and possible long-term problems. Only one subsequently elected for mid-trimester fetal blood sampling, because of a past history of miscarriages and threatened miscarriage in the pregnancy to be tested.

The method for transcervical CVS used was as we described originally (Ward *et al.* 1983) except that the linear array ultrasound machine was replaced in 1984 by an ATL sector scanner.

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Three operators were involved: R.H.T.W. (146 patients), P.K. (12 patients), J.H. (1 patient) and D.M. (4 by transabdominal sampling, see below). Our initial experience suggested that the fetal loss and failure rates were related to late sampling (after 11 weeks) and repeated aspiration attempts, so after a series of 36 pregnancies we decided that no more than two attempts should be made, and that transcervical aspiration would not be offered after 10 completed weeks gestation. After 152 prenatal diagnoses in this series, we have offered transabdominal CVS using a double needle technique (Maxwell & Lilford 1986) for four patients presenting between 11 and 14 weeks, including one who had successful transcervical aspiration, to confirm that the fetus was a homozygote. Thus since mid 1986, fetoscopic blood sampling has been rendered unnecessary for prenatal diagnosis of the haemoglobinopathies in all but the very late attenders or when diagnosis was not possible using DNA.

Occasionally patients who presented for counselling were found to have non-viable pregnancies. They were offered uterine evacuation and are not included in this series. When there were reasons for concern such as vaginal bleeding, CVS was delayed whenever time allowed. In some patients we were obliged to sample before the results of family DNA studies were available and in some others when there was only a 50% chance of diagnosis by CVS and gene-mapping. In these circumstances when diagnosis by gene-mapping was inconclusive, fetoscopic blood sampling was undertaken at 18 weeks gestation.

At the time of CVS, the villi were examined under a dissecting microscope and then removed into fresh medium for immediate transport to the laboratory. The villi were weighed and DNA was prepared and analysed in all the cases at risk of a haemoglobinopathy (Old *et al.* 1986). Fetal karyotyping was done both by direct preparation and culture. Other specimens were analysed by colleagues at St Mary's and Guy's Hospitals, and the Institute of Child Health.

The patients were invited to return for an ultrasound examination and serum alpha fetoprotein estimation at 17 weeks gestation.

Results

Table 1 summarizes the indications for CVS. Two couples were at risk of both beta-thalassaemia and another single gene disorder. Of the 136 patients 22 presented in more than one pregnancy, five women were seen in three pregnancies each.

Table 2 summarizes the mean gestational age at sampling, the mean number of insertions, the weight of villi obtained (for those at risk of a haemoglobinopathy) and the number of failures. It has been our policy to obtain a generous sample to allow for repeat laboratory analyses when appropriate, and with time the number of insertions necessary to yield an adequate sample has decreased. There was a total of five failures, four of which occurred in the first 40 pregnancies (Table 3). In the two pregnancies sampled after 11 weeks failure was due in the main to the fundal placenta. In pregnancy no. 143, diagnosis was not possible with a small sample obtained from a very thin chorion frondosum which itself may possibly explain the subsequent missed abortion. Fetoscopy at 18 weeks gestation was necessary in 11 of the pregnancies tested, usually when DNA analysis had not been able to provide a diagnosis.

An affected fetus was present in 51 of the pregnancies, four of which were lost *before* the diagnosis of an affected fetus was known (including one set of mono-amniotic twins). Of the remaining 47 pregnancies with affected fetuses, 43 were electively aborted including a twin pregnancy with one affected fetus. In this case, the couple decided to continue the pregnancy but were advised by their obstetrician to terminate at 18 weeks because of persistent bleeding. There was one false negative diagnosis of homozygote alpha-thalassaemia resulting in a stillbirth at 28 weeks (Old *et al.* 1986) and three couples with affected fetuses (all with β-thalassaemia) decided to continue their pregnancies.

Two women with unaffected fetuses had either oligohydramnios or persistent vaginal bleeding and requested termination at 18 and 19 weeks respectively. In 113 pregnancies the decision was made to continue (including the three with affected fetuses). Nine pregnancies were subsequently lost before 24 weeks (8%) but six of these were among the first 40 pregnancies giving a fetal loss of 22% (6 of 27) as compared with 3·5% (3 of 86) in the rest of the series.

The aetiology of all 13 fetal losses (including the four in affected pregnancies and two failures) are detailed in Table 4. Trauma was the main cause in six, and in three of them the CVS procedure was at 12·5 weeks or later.

Table 1. Indication for chorionic villus sampling (CVS) by method of diagnosis

Method of diagnosis	Indication for CVS	Number of pregnancies
DNA studies (n = 147)	β-thalassaemia (one pair of twins)	122
	Sickle cell anaemia (one pair of twins)	16
	α-thalassaemia	5
	Hb Lepore/β-thalassaemia	1
	Total haemoglobinopathies	144
	*Cystic fibrosis	2
	Rubella	1
Fetal sexing and DNA studies when appropriate (n = 10)	Adreno-leukodystrophy	1
	Haemophilia	4
	Muscular dystrophy (Becker's or Duchenne's)	7
Fetal sexing only (n = 3)	Coffin–Lowry syndrome (one pair of twins)	1
	Familial hirsutism	1
	Anhydrotic ectodermal dysplasia	1
Biochemical (n = 3)	*Maple syrup urine disease	1
	Argininosuccinaciduria	2

*Indicates a patient at risk of β-thalassaemia also.

Table 2. Details of procedure for chorionic villus sampling*

Pregnancy no.	Mean gestational age (weeks)	Mean no. of insertions	Weight of villi* (mg) Mean (range)	Number of failures
1–40	10·0	2·75	44·8 (5–120)	4
41–80	9·6	1·38	46·5 (11–100)	0
81–120	9·6	1·28	72·8 (3–214)	0
121–163	9·7	1·23	82·5 (4–246)	1

*Weight of villi was only available for pregnancies at risk of haemoglobinopathies.

We remain convinced that uterine perforation did not occur at the time of CVS (patient no. 1) although this was subsequently recorded at dilatation and curettage by the referring gynaecologist.

Infection was considered likely as a factor in three fetal losses. Two of these were in the same patient (pregnancies nos 10 and 106). Diagnosis in the first pregnancy showed that the husband was not the father but in both pregnancies the courses after CVS were similar with intermittent vaginal bleeding with an ultrasonically demonstrated haematoma in one, and miscarriage 7 weeks later. Cervicitis was present on both occasions at CVS and cultures grew a mixture of anaerobes. At the time of the second miscarriage (augmented by oxytocin) there was a fever and on subsequent pathological examination evidence of chorioamnionitis. In a subsequent pregnancy transabdominal CVS was performed and the pregnancy proceeded to term. The other patient (pregnancy no. 24) bled briefly before miscarriage occurred at 22 weeks. The CVS was recorded as difficult but no haematoma formation was noticed subsequently. Within a week clinical examination suggested that miscarriage was inevitable although it did not occur for a further 13 weeks. Chorioamnionitis was suspected but there was no bacteriological confirmation of infection nor any maternal fever. The woman had two subsequent pregnancies treated with transcervical CVS; one ended in a

Table 3. Outcome in pregnancies where chorionic villus sampling failed

Pregnancy no.	Gestational age (weeks)	Site of placenta	Number of attempts	Pregnancy outcome
12	12·6	Fundal	6	Miscarriage
13	9·0	Anterior	3	Fetoscopy, livebirth
16	11·6	Fundal	3	Fetoscopy, livebirth
27	8·4	Posterior	2	Fetoscopy, livebirth
143	8·4	Posterior	2	4 mg villi insufficient for diagnosis, missed abortion 16 days later

Table 4. Fetal losses after chorionic villus sampling (CVS)

Pregnancy no.	Diagnosis	Gestation at CVS (weeks)	Outcome of pregnancy	Aetiology
1	Unaffected	8·5	Missed abortion 4 days later	Trauma
8	Unaffected	12·5	Miscarriage at 16 weeks	Ruptured membranes, trauma
10*	Unaffected	10·5	Miscarriage at 17 weeks	?Infection
11	Affected	10·5	Miscarriage at 11 weeks	Fixed hip and fixed retroverted uterus, trauma
12	No sample obtained	12·5	Miscarriage at 13·5 weeks	Six unsuccessful attempts, trauma
24	Unaffected	8	Miscarriage at 22 weeks	?Infection
30	Unaffected	13	Miscarriage at 14 weeks	?Trauma
41	Affected male twins	11	Missed abortion 2 days later	Three previous miscarriages, risk of Coffin–Lowry syndrome
102	Affected	9	Miscarriage at 10 weeks	Trauma
106*	Unaffected	9·5	Miscarriage at 17 weeks	Infection
141	Affected	10	Miscarriage at 11 weeks	?Ongoing pregnancy at CVS
143	4 mg sample obtained, no diagnosis	8·5	Missed abortion at 11 weeks	?Ongoing pregnancy at CVS
154	Unaffected	10	Miscarriage at 20 weeks	One live child, six subsequent abortions or premature neonatal deaths

*Pregnancies 10 and 106 were in the same patient.

livebirth at term and the other was terminated at an early stage for an affected fetus.

Of the remaining four pregnancy losses, we doubted that all was well at ultrasound examination before CVS in two, even though a living fetus was demonstrated, and both of the other two patients had had significant failures in previous pregnancies.

Apart from the stillbirth of the fetus with homozygous alpha-thalassaemia, there were two perinatal deaths; one occurred after a preterm delivery at 28 weeks in Africa, and the other after cardiac surgery for total anomalous pulmonary venous drainage. Thus of the initial 163 pregnancies (166 fetuses), 102 resulted in the birth of 103 live babies that survived (including three affected with β-thalassaemia).

Discussion

In 1982 CVS was still at the experimental stage but in the 4 years covered by this study it has become accepted as an alternative to second trimester diagnosis for couples with a high genetic risk.

Improved performance of CVS is indicated by the reduced number of attempts required to obtain a satisfactory sample, and the rapid fall in the frequency of failures and fetal losses. We soon came to recognize that the quality of the ultrasound picture is a key factor for successful and safe sampling. For instance, in the early part of this series, the high frequency of pregnancy losses up to 24 weeks and failures coincided with malfunction of the ultrasound equipment which persuaded us to abandon CVS for 6 months until a replacement machine was available. Subsequently the pregnancy losses up to 24 weeks including those of affected fetuses fell to 6 of 123 (5%). This is still above the general rate of 3·5% of fetal loss in predominantly older women reported in the CVS newsletter (Jackson 1987); but the particular features of our service in part account for this. The high-risk group of patients we deal with are highly motivated for early prenatal diagnosis, so even when risk factors (such as a poor obstetric history, threatened abortion, gestational age over 11·5 weeks, or an unfavourable arrangement of the chorionic villi) are present, they persist in requesting early prenatal diagnosis. We now try to resolve some of these difficulties by transabdominal sampling. Secondly, karyotyping is not routinely available for women less than 35 years old, and so is not offered for most in this high-risk series except on an individual basis. Thus we have no information as to whether any of the losses were due to a fetal chromosome disorder. From an early stage we had the impression that when the sample is of uncontaminated villi, the pregnancy prognosis is good, and that the size of the sample is not necessarily related to the subsequent outcome of the pregnancy. Conversely, failure, particularly when decidual tissue was sampled, often caused bleeding and was associated with an increased rate of fetal loss.

Certain positions of the placenta cause difficulty at transcervical CVS, but can often be favourably altered by manipulation of the uterus, or by filling or emptying the urinary bladder. Nevertheless, the acutely retroverted uterus with a posterior placenta, the anteverted uterus with an anterior placenta, and an entirely fundal placenta in a larger uterus are now all recognized as likely to present technical difficulties at transcervical CVS. The complementary role of transabdominal CVS has been evident when patients arrive too late for safe transcervical aspiration, and when relative contraindications are present. However, a posteriorly sited placenta in a fixed retroverted uterus may not be easy to sample transabdominally until into the second trimester.

The simple design of the Portex cannula has stood the test of time. One criticism directed at the plastic catheter is that the end of the catheter 'drifts' when the obturator is removed. However, if the tip of the catheter is advanced slightly into the placental substance, aspiration of villi is invariably successful. The added advantages of sampling well into the main mass of the chorion frondosum are that membrane or decidual damage seems less likely, and that unhealthy villi are rarely obtained. Though a diagnosis has been achieved with as little as 5 mg of chorionic tissue (Old *et al.* 1986), 30–50 mg of villi are desirable for DNA studies and allowance is best made for the occasional laboratory rerun. In the latter half of this series the quantities obtained have usually been above this target, but their removal has not been associated with any adverse consequences. The one false negative diagnosis was due to plasmid contamination of the Southern blot.

The fact that resort to mid-trimester fetoscopic blood sampling is now infrequently necessary for the diagnosis of the haemoglobinopathies is certainly a great improvement for the families at risk. The rate of change to CVS has been determined by the limitations of the DNA methods, the time limits governing CVS and the need for family studies. One of the most important lessons we have learnt is that the transition to early pregnancy diagnosis can be successfully made only if very considerable time and effort are invested in informing patients about the new development in good time, and arranging family studies preferably before they embark on a pregnancy, or immediately on hearing that the woman is pregnant. This requires the availability of a genetic counselling service where pregnant women can be seen at short notice. Those couples at risk of β-thalassaemia with only a 50% chance of diagnosis due to limitations of the current gene-mapping method face a very stressful prospect; however, increasing laboratory expertise (Old *et al.* 1986) means that a complete diagnosis was possible for 70% of couples at risk in 1986 and for 90% in 1987 (up to July).

Our experience of using the cells in amniotic fluid for DNA-based diagnosis was unsatisfactory (Old *et al.* 1986) because the DNA obtained is often degraded and unsuitable for analysis, so we abandoned this approach. However, where the transcervical route is unsuitable because a pregnancy is too advanced (e.g. from 11 to 14 weeks gestation), it turns out that transabdominal CVS can be safely performed, and satisfactory samples for direct DNA analysis or karyotyping can be obtained (D. Maxwell, personal communication). We conclude that CVS in early pregnancy is a prenatal diagnostic technique that should be available to couples with a high genetic risk. Careful counselling, as in all prenatal diagnosis, is essential, particularly as there still appears to be an increased risk of fetal loss compared with mid-trimester methods.

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Does impaired glucose tolerance imply a risk in pregnancy?

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Summary. Of 218 pregnant women with abnormal glucose tolerance by the criteria of the World Health Organization (1985) 81·2% had impaired glucose tolerance and 18·8% gestational diabetes. Gestational diabetic women were of higher parity, more obese, required insulin therapy more often, had more babies weighing >4 kg and had higher fasting plasma glucose than women with impaired glucose tolerance. Women with gestational impaired glucose tolerance were older, of higher parity, more obese and had heavier babies than pregnant women with a normal screening plasma glucose. Compared with women with impaired glucose tolerance, gestational diabetic women were more likely to have abnormality, and more severe impairment of their glucose tolerance test in the puerperium.

Identification of abnormalities in glucose tolerance (GT) by measuring blood glucose during routine antenatal care is widely recommended (Second International Workshop-Conference on Gestational Diabetes Mellitus (SIW-CGD) 1985; Lind 1984; Lind & Anderson 1984), but screening methods and diagnostic criteria vary (Hadden 1985). The SIW-CGD (1985) recommended the use of O'Sullivan criteria for screening and diagnosis. Others recommend the World Health Organization (WHO) Study Group (1985) criteria (Jarrett 1981; Lind 1984; Lind & Anderson 1984). Beard & Hoet (1982) consider that even minor disturbance of carbohydrate tolerance increases the risk to the fetus, and so using the WHO criteria for diabetes mellitus may underestimate the problem. Inclusion of the category of abnormal GT, which is at the upper end of the normal distribution has been questioned (Hadden 1980; Hadden 1985), since it may contribute a very minor increase in fetal risk. The importance of recognizing abnormalities in GT during pregnancy is not only to provide temporary therapy on the assumption that the abnormal glucose state will revert to normal after delivery, but to be aware that this state may persist, reappear after delivery (O'Sullivan 1975) or may be the first recognition of diabetes (Buschard *et al.* 1987). This is especially true in populations with limited access to primary care.

Provision of health care in Saudi Arabia has improved dramatically in the last decade, with primary care gradually being recognized as an important part. But in many areas such specialist services as antenatal clinics perform a primary care duty and a wide range of disease severity (e.g. diabetes mellitus) may first be identified in these clinics.

The prevalence of abnormality in GT during pregnancy in our community is about 10% with 8% gestational impaired GT and 2% gestational diabetes (Al-Shawaf *et al.* 1988). This study aims

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to address the question whether gestational impaired GT implies a risk in pregnancy, and is the risk related to the degree of impairment?

Patients and methods

The antenatal clinic at the King Abdul Aziz University Hospital, Riyadh, a self-referral clinic, sees about 1500 women annually. During a period of 30 months from June 1984 to December 1986, 218 pregnant women were diagnosed to have impaired GT or gestational diabetes. A further 884 women attending the authors' routine antenatal clinic between June 1984 and June 1985 who had normal screening plasma glucose values during pregnancy acted as controls. Screening and diagnosis followed guidelines recommended by WHO (1985) and reported by us previously (Al-Shawaf *et al.* 1987, 1988). Briefly a test was considered positive when a single plasma glucose value was ≥7·8 mmol/l at 2 h after an oral 75-g glucose challenge test. Diagnosis was confirmed by a GT test using a 75-g glucose load and following guidelines recommended by WHO (1985). Glycosylated haemoglobin (HbAl) was estimated in the fasting state before the GT test.

Women with abnormal GT were divided into those with gestational impaired GT (fasting plasma glucose <7·8 mmol/l and the 2-h plasma glucose 7·8–11 mmol/l) and those with gestational diabetes (fasting plasma glucose >7·8 mmol/l or 2-h plasma glucose >11 mmol/l or both).

Women were seen regularly in a combined pregnancy diabetic clinic. Diet was started according to ideal body-weight requirement. Insulin therapy was then instituted arbitrarily if plasma glucose 2 h after a meal was >7 mmol/l.

All women in the programme were requested to have a repeat GT test at 6 weeks postpartum, but only 55 (25·5%) returned for this test after delivery. Newborn infants were examined for Apgar scores at 1 and 5 min, birthweight and congenital abnormalities; they were observed for at least 48 h in the neonatal care unit before discharge.

Plasma was assayed for glucose by a glucose hexokinase method using an Auto analyser Technician SMAC. HbAl was assayed by haemoglobin electrophoresis. To control errors in method, internal (Technician multi-system control) and external quality control (Middle East quality control system) procedures were used. The coefficient of variation for glucose estimation for the low and high target values was 3% and 4% respectively, while for the HbAl it was 6%.

Statistical analysis

Data are presented as means (SD), (SEM) and 95% confidence interval (CI). Significance of difference between means was evaluated by unpaired CI testing at 95% (Gardner & Altman 1986), χ^2-test, Mann–Whitney *U*-test and Fisher's exact probability test as appropriate. All *P* values were two sided.

Results

Among the 218 women in the study, 177 (81·2%) had a diagnosis of impaired GT and 41 (18·8%) had gestational diabetes. The difference in the mean age (Table 1) between the impaired GT and diabetic groups was 1·4 years (95% CI −2·4 to +5·2; t = 1·5, P>0·1); and between the impaired GT and control groups 4·2 years (95% CI +2·5 to +5·9, t = 9·2, P<0·001). The parity distribution is shown in Table 2. The *Z* value by the two-tailed Mann–Whitney test for the parity distribution between the impaired GT and dia-

Table 1. Age distribution in the study groups

		Age (years)				
Patient group	*n*	Mean	SD	SEM	95% CI	*P*
Control	884	25·2	5·1	0·4	24·4 to 26·0	
Impaired GT	177	29·4	5·6	0·4	28·6 to 30·2	<0·001*
Diabetes	41	30·8	5·3	0·8	29·1 to 32·5	>0·1**

GT, Glucose tolerance.

* Impaired GT compared with control group.

** Diabetes compared with impaired GT.

Table 2. Parity distribution in the study groups

		Parity						
		0		1–4		5		
Patient group	*n*	*n*	(%)	*n*	(%)	*n*	(%)	*P*
Control	884	221	(25·0)	569	(64·4)	94	(10·6)	
Impaired GT	177	26	(14·7)	90	(50·9)	61	(34·5)	<0·001*
Diabetes	41	2	(4·9)	19	(46·3)	20	(48·8)	<0·04**

Results are numbers of women (%).
GT, Glucose tolerance.
* Impaired GT compared with control group.
** Diabetes compared with impaired GT.

betic groups was 2·05 (P = 0·04), and between the impaired GT and control groups it was 5·60 (P<0·001).

Of the 218 women in the study 67 (30·7%) had a family history (first degree relative) with diabetes mellitus. No statistically significant difference was found in the prevalence of familial diabetes mellitus between the impaired GT and diabetic groups (55/177 *vs* 12/41, χ^2_1 = 0·173, P>0·5). There were twice the number with a maternal history of diabetes than with a paternal history in the impaired GT group (36 *vs* 19) and similarly in the diabetic group (8 *vs* 4), but this was not statistically significant. In the control group 163 (18·4%) women had a family history of diabetes (study group *vs* control χ^2 = 15·2710, P<0·001; impaired GT *vs* control χ^2 = 13·6568, P<0·001); but no difference was present between maternal (69) *vs* paternal (55) family history.

The mean gestational age at the initial visit for all the study group was 17·7 weeks (SD 9·2, 95% CI 16·5 to 18·9) which was not significantly different from that in the control group of 17·1 weeks (SD 8·9, 95% CI 16·5 to 17·7). The mean gestational age at diagnosis of abnormal GT was 24·1 (SD 9·4, 95% CI 22·9 to 25·3). The χ^2_2 for diagnosis by trimester (first, second and third) of pregnancy, between the impaired GT and diabetic groups was 0·4354, P>0·5.

Table 3 shows the body mass index (BMI) calculated as weight/height² (kg/m²), for the women who were initially seen in the first trimester. The difference in the mean BMI between the diabetic and impaired GT groups was 2·4 (95% CI −4·2 to +9, t = 1·58, P>0·1) and between the impaired GT and control groups 3·0 (95% CI +0·8 to +5·2, t = 4·97, P<0·001). The prevalence of women who had a BMI ⩾30 in the first trimester was comparable between the diabetic and impaired GT groups (χ^2_1 = 2·01, P>0·1). Compared with the control group, women with impaired GT were more likely to have a BMI ⩾30 (χ^2_1 = 18·31, P<0·001). Insulin therapy was required more often in the diabetic group than in the impaired GT group (51·2% compared with 5·6%), this difference was significant (χ^2 = 52·99, P<0·001) and was expected as the initiation of therapy was based on blood glucose levels.

Of the 218 women with abnormal GT 18 (8·2%) developed raised blood pressure with or

Table 3. Body mass index (BMI) in the first trimester

		BMI (kg/m²)				
Patient group	*n*	Mean	SD	SEM	95% CI	BMI ⩾ 30 *n* (%)
Control	429	25·9	4·9	0·5	25·5 to 26·3	85 (19·8)
Impaired GT	98	28·9	5·5	0·6	27·7 to 30·1*	40* (40·8)
Diabetes	13	31·3	5·1	1·4	28·3 to 34·4**	9 (69·2)**

GT, Glucose tolerance.
* Impaired GT compared with control P<0·001.
**Diabetes compared with impaired GT P>0·1.

without other symptoms of pre-eclampsia, they comprised 14 women with impaired GT and 4 with diabetes (χ^2_1 = 0·0052, *P*>0·5). In the control group 44 (5·0%) women developed raised blood pressure, which was not statistically different from the study group (χ^2_1 = 2·9515, *P*>0·05). There was no difference in the mode of delivery (spontaneous *vs* instrumental *vs* caesarean section) between the diabetic and impaired GT groups (χ^2_1 = 4·09, *P*>0·1). The rate of caesarean section in our unit is 9%, which is comparable to the rate in the impaired GT group (6·8%) but lower than that in the diabetic group (14·6%). Caesarean sections were mainly performed for obstetric indications.

The difference in mean birthweight between the impaired GT group (mean 3·427 kg, SD 0·47, 95% CI 3·37 to 3·49) and the diabetic group (mean 3·569 kg, SD 0·54, 95% CI 3·40 to 3·74) was 0·142 (95% CI −0·16 to +0·50, *t* = 1·56, *P*>0·1). The difference in mean birthweight between the impaired GT and control groups (mean 3·320 kg, SD 0·44, 95% CI 3·28 to 3·36) was 0·107 kg (95% CI −0·04 to +0·25, *t* = 2·789, *P*<0·01). Heavy babies (≥4 kg) were more frequent in the diabetic group than in the impaired GT group (χ^2_1 = 5·97, *P*<0·02), but the frequency was comparable between the impaired GT and diabetic groups (χ^2_1 = 2·998, *P*>0·05) (Table 4).

The ratio of male to female infants was 1·6 in the diabetic group, 1·04 in the impaired GT group and 0·9 in the control group (χ^2_2 = 2·54, *P*>0·1). No difference in the Apgar score was present at 1 or 5 min between the groups studied (Table 4). The rate of major congenital malformation in the study group was 22·9/1000 births and in the control group 11·3/1000 births (Table 4). The perinatal mortality rate was 9·2/1000 births (two infants) in the study group and 9·1/1000 births (eight infants) in the control group.

The GTT results showed that the mean fasting plasma glucose value was 5·4 mmol/l (SD 0·07, SEM 0·05, 95% CI 5·3 to 5·5) in the impaired GT group and 6·7 mmol/l (SD 1·3, SEM 0·2, 95% CI 6·1 to 7·3) in the diabetic group. The difference between the means was 1·3 mmol/l (95% CI +0·73 to +1·87, *t* = 6·20, *P*<0·001). None of the women in the impaired GT group had a fasting plasma glucose value ≥7·2 mmol/l whereas 16 diabetic women had a value ≥7·2 mmol/l. The mean glycosylated haemoglobin level was 7·2% (SD 1·7, SEM 0·2, 95% CI 6·6 to 7·6) in the impaired GT group and 7·7% (SD 1·9, SEM 0·4, 95% CI 6·9 to 8·5) in the diabetic group. The difference in the means was 0·5% (95% CI −0·09 to 1·90, *t* = 1·24, *P*>0·1). Only 55 women (25·2%) of all the study group had a GTT at 6 weeks postpartum (Table 5), the rest were lost to follow-up. Persistent abnormal GT was found in 10 of 15 (66·7%) women with gestational diabetes and in 12 of 40 (30%) with gestational impaired glucose tolerance (χ^2 = 4·39, *P*<0·05). The GTT was indicative of diabetes mellitus in six of those who had gestational diabetes and in one woman with gestational GT, the other abnormal GTT were impaired GT (Fisher's exact test *P* = 0·009).

Discussion

The prevalence of diabetes in the general population in Saudi Arabia is 4·5%, with non-insulin-dependent diabetes comprising 80·8% of all diabetics (A. A. Hagroo *et al.*, H. H. Fatani *et al.* unpublished data). Primary care is limited in Saudi Arabia which may explain the high preva-

Table 4. Apgar score and congenital malformation

Patient group	*n*	Birthweight ≥4 kg	Apgar score <6		Congenital malformation	
			1 min	5 min	Minor	Major
Control	884	59 (6·7)	144 (16·2)	14 (1·6)	10 (1·1)	10 (1·1)
Impaired GT	177	19 (10·7)	29 (16·4)	1 (0·6)	2 (1·1)[a]	4 (2·3)[b]
Diabetes	41	11 (27)	10 (24·4)	1 (2·4)	—	1 (2·4)[c]

Results are numbers of infants (%).

GT, Glucose tolerance.

[a] (1) Congenital dislocation of hips; (2) syndactylia.

[b] (1) Intestinal obstruction: (2) exomphalous; (3) hydrocephalus; (4) trisomy 21.

[c] Congenital heart disease.

Table 5. Glucose tolerance (GT) test results at 6 weeks after delivery

		Diagnosis at 6 weeks postpartum		
Antenatal diagnosis	*n*	Normal	Impaired GT	Diabetes
Impaired GT	40	28 (70)	11 (27·5)	1 (2·5)
Diabetes	15	5 (33·3)	4 (26·7)	6 (40)
Total	55	33 (60)	15 (27·2)	7 (12·8)

lence (10%) of abnormal GT first recognized in pregnancy (Al-Shawaf *et al.* 1987).

The present study showed some differences between gestational diabetics and women with impaired GT regarding parity, obesity, need for insulin treatment, birth of heavy babies and higher fasting plasma glucose values. Women with impaired GT also differ from normal pregnant women (with 2-h plasma glucose <7·8 mmol/l after a 75-g glucose load) in age, parity obesity and heavier infants. The frequency of major fetal congenital anomalies in women with abnormal GT was twice that in the control group (2·3% *vs* 1·1%).

Pettitt *et al.* (1985) reported a lower rate of large-for-gestational-age infants, pre-eclampsia, and caesarean section in women who had impaired GT than in women with diabetes or those with gestational diabetes. Their women with impaired GT had a higher frequency of these complications than pregnant women with normal GT, albeit less than diabetics. They supported the hypothesis (Freinkel 1980) that minor metabolic disturbances in pregnancy affect the anthropometric and metabolic status of the offspring many years later. Pregnant women who had a plasma glucose value between 5·6 and 9·1 mmol/l at 2 h after a 100-g glucose load had a significantly increased rate of large babies, congenital abnormalities, pre-eclampsia and caesarean section (Tallarigo *et al.* 1986), with the highest rate in those with levels between 6·6 and 9·1 mmol/l (120 and 164 mg/dl).

Landon & Gabbe (1985) suggested that class A gestational diabetics (fasting glucose value 5·8 mmol/l and 2 h postprandial <6·7 mmol/l) are at lower risk, and they observed no difference in the frequency of large babies in class A and class AB (insulin requiring) diabetic women. Routine insulin treatment of pregnant women with mildly impaired GT is unnecessary, although 14% may require it in addition to a controlled diet (Persson *et al.* 1985). In the present study 10 women (5·6%) with impaired GT were prescribed insulin, significantly fewer than in the diabetic group where about half were considered to require insulin.

Only 55 women (25·2%) had a GT test in the puerperium. The overall prevalence of persistent abnormal GT was 40% (22/55) at 6 weeks postnatal, and women with gestational diabetes were more likely to have a persistent abnormality which was more often diabetes mellitus than impaired GT. Farrell *et al.* (1986) reported that about 33% of their gestational diabetic women failed to attend their 3 months follow-up appointment and Grant *et al.* (1986) reported that only 39·1% had a GT test between 1 and 12 years after the index pregnancy. The cumulative prevalence of abnormal GT was 33·3% after 1 year (Farrell *et al.* 1986), and 18·8% between 1 and 12 years from the index pregnancy (Grant *et al.* 1986). Freinkel & Metzger (1985) found abnormal GT at the postpartum assessment in 57% of the women who had had gestational diabetes mellitus. In Aberdeen 10–15% of gestational diabetics had a persistent abnormality in the puerperium, and 35% had shown abnormal i.v. GT test results during a follow-up period of up to 22 years (Stowers *et al.* 1985). O'Sullivan (1975) in a long-term study projected an incidence of diabetes mellitus of 60%, and the prevalence was 55·6% among women followed up to 5 years after diagnosis (Mestman *et al.* 1972). All these reports use different diagnostic criteria making comparison somewhat difficult.

Significant predictive factors for abnormal GT in the subsequent years after diagnosis of gestational diabetes have been shown to include a fasting plasma glucose value of >5·8 mmol/l, age (Metzger *et al.* 1985; Stowers *et al.* 1985), an abnormal GT test after delivery and recurrence in subsequent pregnancies (Grant *et al.* 1986), a plasma glucose value of ≥11 mmol/l at 2 h after a 100-g glucose load in pregnancy (Mertzger *et al.* 1985). Buschard *et al.* (1987) showed that most women who develop insulin-dependent diabetes during pregnancy had type 1 diabetes.

Some of our patients who had persistent abnormality at 6 weeks postpartum, consistent with diabetes mellitus, may have been diabetic before the index pregnancy.

Impaired GT in pregnancy has been reported to have adverse effects on the pregnancy (Pettitt *et al.* 1985), and also on the anthropometric and metabolic status of the offspring many years later (Freinkel 1980; Pettitt *et al.* 1985). Our findings support the view that gestational impaired GT as defined by the WHO (1985) does constitute a risk to pregnancy, but less than that associated with gestational diabetes.

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Comparison of four continuously administered progestogen plus oestradiol combinations for climacteric complaints

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Summary. Sixty women with climacteric complaints who had not menstruated for at least 1 year were randomly allocated to receive one of four hormonal replacement regimens. All four formulations were administered daily and continuously and each contained 2 mg of micronized oestradiol-17β in combination with either norethisterone acetate 1 mg (group A) or 0·5 mg (group B) or megestrol acetate 5 mg (group C) or 2·5 mg (group D). The clinical efficacy was the same although the alleviation of vasomotor symptoms was somewhat slower in those women receiving preparation A. The endometrium was atrophied in nearly all biopsies. Irregular uterine bleeding was almost entirely confined to the earlier phase of the study and was substantially less with the formulation containing 1 mg norethisterone acetate. It is concluded that a continuous oestradiol–progestogen combination can be used for long-term treatment of climacteric complaints in postmenopausal women and that after 4 months the clinical efficacy is the same irrespective of the type and dose of progestogen administered.

Both oestrogens and progestogens have been advocated for the treatment of climacteric complaints (Callantine *et al.* 1975; Schiff *et al.* 1980). A combination of both sex steroids has been used both in cyclical and in sequential regimens. The use of progestogens in each treatment cycle has been shown to decrease the occurrence of endometrial hyperplasia (Paterson *et al.* 1980; Whitehead *et al.* 1981) and there is evidence that progestogens also may protect against the development of endometrial cancer related to the intake of oestrogen alone (Gambrell *et al.* 1980). The duration of the progestogen treatment rather than the dose seems to be important for preventing endometrial hyperplasia (Paterson *et al.* 1980). Recently, a new preparation was introduced which contains 2 mg oestradiol-17β and 1 mg norethisterone acetate given as one tablet daily without interruption. This combination was found to be suitable for postmenopausal women because of the lack of bleeding disturbances and the finding of an atrophic corpus mucosa (Staland 1981; Mattsson *et al.* 1982).

Because of their role as biochemical predictors of cardiovascular morbidity, metabolism of lipids and lipoproteins has been a focus of interest during the last decade. Progestogens given as a complement to the oestrogen compound in the treatment of climacteric symptoms may increase the risk of atherosclerotic disease. There is

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debate whether a progestogen derived from 19-nortestosterone or 17α-hydroxyprogesterone should be used as hormone replacement therapy in the postmenopausal period (Silfverstolpe *et al.* 1979; Krauss 1982). As a first step we decided to investigate whether progestogens in different doses are equally effective from the clinical point of view. The present investigation was undertaken to assess the clinical effects of four different progestogen combinations in 60 postmenopausal women on bleeding patterns, vasomotor symptoms and the histopathology of the endometrium. These factors are of great clinical impact for the patient and might differ between various oestrogen–progestogen formulations.

Patients and methods

Sixty postmenopausal women with climacteric complaints, and with a mean age of 55·4 years (range 44–68) attending our outpatient clinic, were randomly allocated to one of four treatment groups using a randomization code. All women were postmenopausal, having had their last menstrual period at least 1 year preceding the study. The women in the four groups did not differ in pretreatment values of age, weight, blood pressure, and years since menopause as tested by analyses of variance (Table 1). Approval for the study was obtained from the ethics committee, faculty of medicine, University of Göteborg, and all the patients gave their written informed consent.

Hormone therapy

No hormone treatment had been given for at least 6 weeks before the study. Women were treated with a continuous oestrogen–progestogen regimen consisting of 2 mg oestradiol-17β (E2) daily in combination with either norethisterone acetate (NETA), a 19-nortestosterone derivative, 1 mg (group A) and 0·5 mg (group B), or megestrol acetate (MEGA), a 17α-hydroxyprogesterone derivative, 5 mg (group C) and 2·5 mg (group D).

Of the 60 women, 48 were still taking part in the study after 1 year of treatment. Seven out of 12 women stopped treatment because of bleeding disturbances. From the numbers shown in Fig. 1, one can calculate how many of the women remained in the study after 4 and 12 months of treatment in the different groups.

Design and methods

Each patient was examined once before treatment and again after 4 and 12 months of treatment. A general physical examination, including body weight and blood pressure measurements, as well as a gynaecological examination with uterine curettage was performed at each visit. Endometrial specimens were obtained with a Vabra curette or a Mi-Mark helix. All specimens were fixed in neutral formalin, embedded in paraffin and sectioned at several levels. Sections were stained with Van Gieson and examined with the microscope for signs of atypia, hyperplasia, malignancy or other pathological conditions.

The total number of sweats and hot flushes during 24 h were recorded by each patient before treatment and then after 1, 4, and 12 months of treatment according to a special protocol. The women were asked to register each hot flush and sweating by a mark in the protocol. Side-effects and bleeding patterns were recorded continuously during the 1-year study period in a daily calendar book kept by each patient. The type of

Table 1. Baseline characteristics of four groups of women before starting hormone replacement therapy

Group	*n*	Age (years)	Height (cm)	Weight (kg)	Blood pressure (mmHg) Systolic	Blood pressure (mmHg) Diastolic	Years since menopause
A	15	55·5 (0·9)	165 (1·6)	70·1 (2·6)	141 (4)	85 (3)	5·5 (0·7)
B	15	56·5 (1·3)	162 (1·7)	71·9 (3·2)	144 (5)	88 (2)	5·3 (1·1)
C	15	54·1 (1·4)	161 (1·6)	68·1 (2·8)	136 (5)	85 (2)	6·1 (1·5)
D	15	55·3 (0·8)	162 (2·5)	65·0 (2·5)	139 (5)	86 (2)	5·9 (0·9)

Results show mean (SE) values.

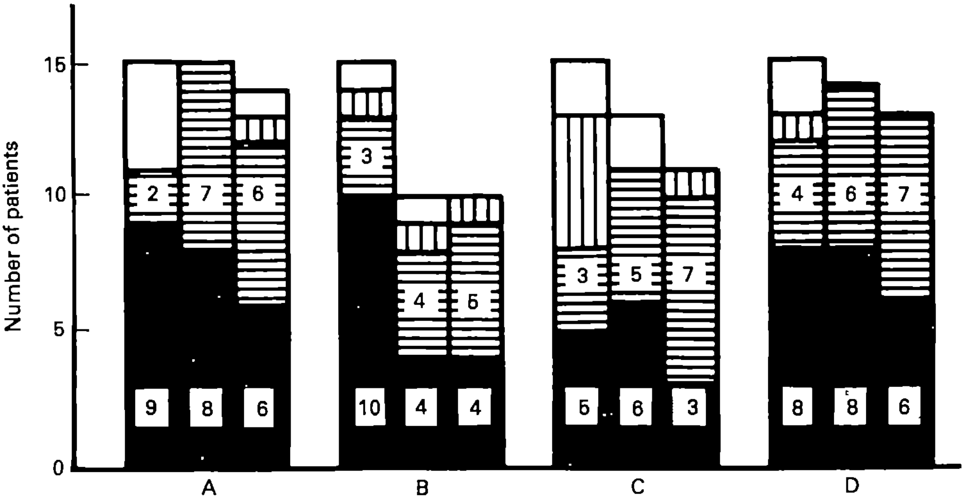

Fig. 1. Endometrial histopathology in four groups of women treated with different continuously administered oestradiol–progestogen combinations before treatment (left bar), after 4 months (middle bar) and after 12 months of treatment (right bar). The height of the bars and the figures inside the bars indicate the number of women. ■, Atrophic; ▤, surface epithelium (scanty specimen); ▥, weak secretory or proliferative; □, cervical os too narrow for sampling.

uterine bleeding was characterized as menstrual-like, slight, or spotting (bloody discharge).

Statistics

Conventional methods were used for calculation of the mean, standard deviation, and standard error of the mean. Correlations were tested by Spearman's rank correlation test. Analysis of variance (ANOVA) was used for assessing differences between groups and within groups. Wilcoxon's signed rank test was used to test differences within groups during treatment. The Mann–Whitney test was used to assess differences between groups. A χ^2-test was used to analyse differences in endometrial histology between the groups.

Results

Blood pressures were within normal values and no differences were found between the groups. No significant changes occurred during treatment.

Vasomotor symptoms

Table 2 shows the mean number of hot flushes during treatment. Before treatment, an average of 10 daily episodes of vasomotor complaints were registered by each patient. After 1 month of treatment, such episodes were reduced by more than 80% in treatment groups B, C, and D, while the reduction was somewhat less in group A. A further and similar decrement of vasomo-

Table 2. Number of hot flushes during 24 h in four groups of women before treatment and during continuous hormone replacement therapy

Group	Before treatment	1 month	4 months	12 months
A	10·0 (2·0)	2·1 (0·9)	0·2 (0·2)	0
B	11·1 (2·8)	1·3 (0·4)	0·2 (0·1)	0·9 (0·9)
C	8·2 (1·7)	0·3 (0·3)	0	0
D	10·6 (2·3)	1·6 (0·7)	0	0

Results show mean (SE) values.
All measurements were significantly different from baseline values ($P<0{\cdot}01$).

tor symptoms was seen in all groups throughout the treatment period. The reduction in the mean number of sweats during treatment was similar to that in hot flushes. After 1 month of therapy there was a significant difference in the mean number of sweats and hot flushes between treatment groups A and C ($P<0{\cdot}05$).

Histopathology

The type of endometrial histology before treatment and after 4 and 12 months of treatment is depicted in Fig. 1. There were no obvious differences between the four treatment groups (χ^2-test). The aim was to obtain endometrial specimens from all remaining patients after 12 months of treatment. However, at this time it was impossible to obtain endometrial tissue in one woman with a narrow cervical canal and she did not consent to a conventional dilatation and curettage under general anaesthesia. Hyperstimulation of the endometrium was not found in any of the women.

Uterine bleeding patterns

About 80% of the days with bleeding in all groups occurred during the first 4 months of treatment, while about 10% occurred during the next 4-month period and 10% during the last third of the treatment year. Table 3 shows the mean number of days with bleeding in the first 4 months of treatment. There were significant differences between treatment groups A and B and between treatment groups A and D after 1 month of treatment, $P<0{\cdot}01$. During the first 4 months of treatment the mean number of days with bleeding for group A was 8·1 (SE 5·2), group B 23·1 (SE 5·6), group C 15·5 (SE 5·6), and group D 22·7 (SE 5·6).

Table 4 shows the different types of bleeding during the 12 months of treatment in three time periods of 4 months each. In group A more than 50% of the women were completely free from bleeding during the first 4 months and 86% for the last 4 months (Table 4). Nearly all women in group B had some kind of bleeding during the first 4 months of treatment (93%). No correlation was found between the frequency and type of uterine bleeding and body weights using single regression.

Side-effects

Of the 12 women who stopped treatment, seven did so because of irregular uterine bleeding.

Table 3. Uterine bleeding patterns in four groups of postmenopausal women during the first 4 months of continuous oestradiol–progestogen treatment*

Group	Treatment period (month)	No. of days with bleeding mean (SE)	Type of bleeding as a percentage of all bleeding days in the 4 months of treatment		
			Menstrual (%)	Slight (%)	Spotting (%)
A	1	2·5 (1·0)	7	11	14
	2	2·3 (1·0)	9	18	4
	3	2·2 (1·0)	1	14	14
	4	0·6 (1·0)	0	4	4
B	1	7·1 (2·2)	19	12	8
	2	8·1 (2·5)	6	16	13
	3	4·3 (2·4)	2	13	7
	4	4·6 (2·5)	0	3	2
C	1	4·3 (1·9)	9	9	10
	2	6·8 (1·9)	11	14	10
	3	3·6 (2·0)	0	8	9
	4	4·3 (2·0)	2	10	8
D	1	8·3 (2·0)	7	10	17
	2	6·4 (2·0)	2	4	21
	3	5·1 (2·0)	0	7	14
	4	4·2 (2·0)	0	6	12

* See Table 4 for details of treatment in the four groups.

Table 4. Uterine bleeding patterns in four groups of postmenopausal women during 1 year of continuous oestradiol–progestogen treatment*

Group	Treatment months	No. of days with bleeding mean (SE)	Type of bleeding as a percentage of all bleeding days in each 4-month treatment period: Menstrual (%)	Slight (%)	Spotting (%)	Percentage of women without any bleeding (%)
A	1–4	8·1 (5·2)	17	47	36	53
	5–8	2·7 (1·7)	0	66	34	71
	9–12	1·3 (1·0)	0	16	84	86
B	1–4	23·1 (5·6)	24	40	36	27
	5–8	1·8 (0·9)	0	19	81	64
	9–12	3·1 (3·3)	0	0	100	91
C	1–4	15·5 (5·6)	7	46	47	23
	5–8	1·5 (0·8)	20	33	47	69
	9–12	0·8 (0·5)	0	63	37	85
D	1–4	22·7 (5·6)	9	33	58	7
	5–8	5·6 (2·4)	1	20	79	50
	9–12	2·6 (2·1)	0	15	85	77

* The patients were given 2 mg oestradiol-17β in combination either with norethisterone acetate, 1 mg in group A and 0·5 mg in group B, or megestrol acetate, 5 mg in group C and 2·5 mg in group D.

Four patients complained of depression and irritability while one woman stopped medication because of a deep venous thrombosis. She stopped medication, was referred to hospital and received conventional anticoagulation therapy. Furthermore, in one patient with a hip fracture, a venous thrombosis was diagnosed in the same leg. However, this patient was not advised to stop hormone medication but was treated with anticoagulants for 8 weeks; she had no further problems.

Discussion

Withdrawal bleeding with intermittent hormone replacement therapy may be annoying to women in the postmenopausal period. Therefore a continuous oestrogen–progesterone therapy was introduced a few years ago aimed at abolishing this disadvantage. In previous reports we suggested that this kind of therapy would be most suitable for postmenopausal women (Mattsson *et al.* 1982; Mattsson & Samsioe 1985). In the present study, all the women were at least 1 year postmenopausal. In the four treatment groups all women took 2 mg oestradiol-17β with two different doses of norethisterone acetate or megestrol acetate. The doses were kept constant during the follow-up period of 1 year in all groups. Other investigators have tried increasing the progestogenic potency after some time of treatment in order to obtain amenorrhoea in all those treated (Magos *et al.* 1985).

A marked reduction in vasomotor complaints was encountered quite soon in all treatment groups. However, in a number of patients in the group given 1 mg norethisterone acetate, sweats were not alleviated to the same degree as in the other groups after 1 month of treatment. There was a statistically significant difference in the mean number of hot flushes after 1 month of therapy between treatment groups A and C ($P<0{\cdot}05$). This effect may be due to the anti-oestrogenic effect exerted by norethisterone acetate. It might also be due to the lack of additive effects, as have been described with medroxyprogesterone acetate, which is a 17α-hydroxyprogesterone like megestrol acetate. Although the symptoms were relieved more rapidly in groups B, C and D, the overall clinical impression was of adequate relief in all the groups during the following months, so that all preparations seemed clinically useful.

Most of these postmenopausal women had an atrophic endometrium before treatment. During treatment, almost all the endometrial specimens were either atrophic or inadequate for histopathological study. In our experience, when outpatient procedures fail to obtain enough tissue for assessment, curettage under anaesthesia shows that the endometrium is atrophic. In two patients slight secretory changes were found in the endometrium after 12 months of treatment. No hyperplasia was detected. Our study suggests that the combination of oestradiol with various progestogens at different doses taken continuously minimizes endometrial growth and may protect the endometrium from malignant transformation. Similar findings have been presented by others (Paterson *et al.* 1980; Magos *et al.* 1985) when progestogens were added for at least 12 days in each treatment month.

Irregular bleeding occurred during the first treatment months in all groups, although significantly less ($P<0{\cdot}01$) in women treated with 1 mg norethisterone acetate than in treatment groups B and D. Both the amount of bleeding and the number of days with bleeding decreased gradually during the first 4 months of treatment in all groups. During the last 8 months of treatment bleeding occurred infrequently and was mostly characterized as spotting. Two patients had copious bleeding and both stopped treatment after a few months. One of these patients had a submucous fibromyoma and she was treated surgically. The other woman had previously tried several oestrogen–progestogen combinations, but had experienced continuous bleeding of unknown cause with all of them.

More than 50% of all women given 1 mg norethisterone acetate had no uterine bleeding at all. Other oestrogen–progestogen preparations administered as cyclical or sequential regimens were reported to induce sheddings in more than 90% of all patients (Gambrell *et al.* 1980). It was previously considered necessary to induce endometrial shedding during hormone therapy in the postmenopausal period to guarantee a healthy endometrium. In this study 77–91% of the women still in the study were amenorrheic during the last third of the treatment period, and no abnormal endometrial specimen was found.

It is unknown why some patients and not all develop uterine bleeding on the same kind of treatment. In fertile women treated with medroxyprogesterone acetate for contraception there was no relation between bleeding patterns and serum levels of oestradiol or medroxyprogesterone acetate (Johannisson & Landgren 1980). It has been proposed that frequent bleeding in some women may be due to anatomical variations, vascular disturbance or local abnormal haemostatic mechanisms in the uterus (Paton *et al.* 1980). Differences in the metabolism of the therapeutic steroids may also be important. It would be of great benefit to be able to detect those women predisposed to irregular bleeding before starting hormone replacement therapy. In the present study no correlation was found between body weight and the total days with bleeding. Neither did the time elapsed after menopause correlate with bleeding patterns (Spearman's rank correlation test).

During the first months of treatment bleeding problems occurred least often in group A. Therefore, we suggest that continuous oestradiol–progestogen replacement should comprise a relatively high potency of progestogens. It may be possible to reduce the amount of progestogen during the course of treatment. This approach is in contrast with that of Magos *et al.* (1985). They successively introduced higher doses of progestogens during the course of treatment to obtain amenorrhoea in all patients. However, they also reported a drop-out rate of about 35%, mostly because of bleeding problems. We believe that it is of great importance to reduce the number of episodes of bleeding at the beginning of treatment, as is demonstrated by the good compliance in group A in this study. The progestogen type and dose may affect lipid metabolism and coagulation differently. Long-term effects on these variables are important factors when deciding which preparations are to be recommended for substitution therapy. It is important to inform the patients of the possibility of uterine bleeding, especially during the first period of treatment, to avoid an unnecessary drop-out. Furthermore, it is suggested from the present data that most bleeding stops within a few days and does not warrant an endometrial curettage.

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Vaginal cones: a conservative method of treating genuine stress incontinence

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Summary. A set of weighted vaginal cones designed to exercise the pelvic floor muscles was used by a group of 39 premenopausal patients with genuine stress incontinence who were awaiting corrective surgery. The effectiveness of the exercises in reducing urinary loss was assessed with a standard urine pad test before and after 1 month of training. Of the 30 women who completed 1 month of the exercises 70% felt they were improved or cured and 90% found it an acceptable method of treatment. Of the 30 women only 11 (37%) opted for surgery after training. There was a highly significant correlation ($P<0{\cdot}001$, $r_s = -0.21$) between decreased urine loss and increase in retained cone weight.

An initial conservative approach to the problem of genuine stress incontinence using pelvic floor exercises has the advantage that it may obviate the need for surgery. Commonly, pelvic floor exercises are monitored using a perineometer (Kegel 1949). This, however, does not allow distinction to be made between contraction of pelvic floor and abdominal wall muscles, both being registered on the meter as an increase in pelvic floor activity; patients therefore falsely believe they are improving the strength of their pelvic floor muscles. It has been shown that normal women can be trained to contract their pelvic floor muscles using weighted cones retained in the vagina (Plevnik 1985). This study looks at the efficacy of this new method in the management of genuine stress incontinence.

Patients and methods

Thirty-nine premenopausal patients were enrolled: nine did not complete the course and complete results for 30 are presented. The average age was 38 years (range 18–47) and average weight was 63·4 kg (range 51–100). Four patients were nulliparous, 10 had given birth to one child and 25 had had two or three children each. All patients had stable cystometry and demonstrable stress incontinence. Previous bladder neck surgery and current vaginal or urinary tract infection were criteria for exclusion.

Vaginal cones

The cones are in sets of nine of equal shape and volume, but of increasing weight from 20 to 100 g (Fig. 1). They are composed of differing proportions of perspex and stainless steel with a nylon thread which facilitates removal. A cone is inserted into the vagina, base uppermost, and the patient attempts to retain this. The tapered lower portion rests on the pelvic floor and the

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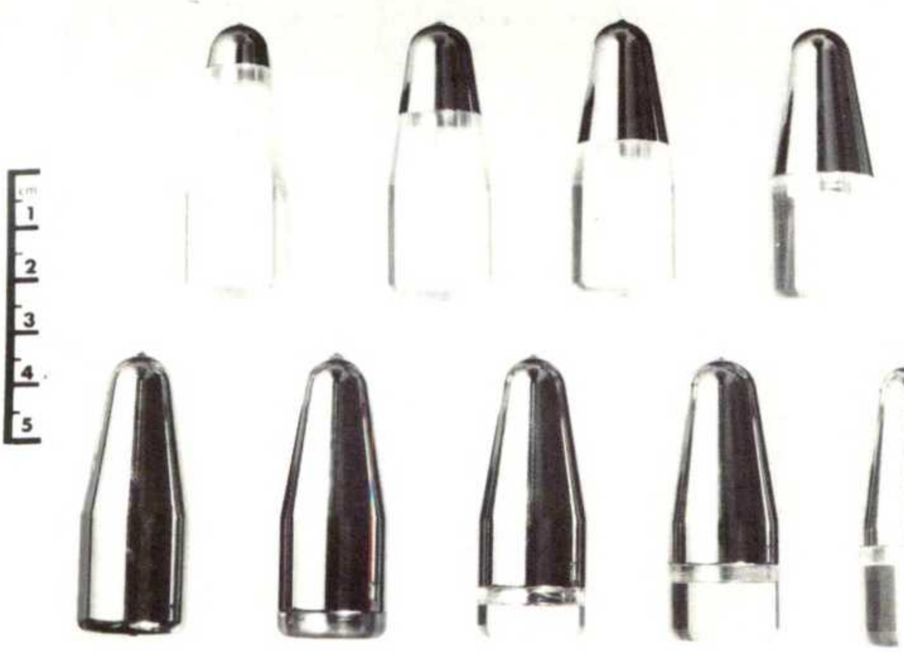

Fig. 1. A set of vaginal cones.

diagram shows how increased intra-abdominal pressure will merely enhance the sensory feedback which makes the pelvic floor contract around the cone, retaining it in the vagina (Fig. 2).

Assessment before training

The patient's urine loss was assessed before treatment using a standard extended pad test which involves the patient drinking 500 ml sodium-free fluid and wearing a pre-weighed sanitary towel whilst exercising with a full bladder. The towel is reweighed after 1 hour and a urine loss of >2 g is accepted as clinically significant. With the patient walking, the weight of the cones was assessed over a period of 1 min per cone. The heaviest weight retained without voluntary holding was called the passive weight and taken to represent resting pelvic floor tone. The heaviest weight retained with voluntary pelvic floor contraction was termed the active weight. Each patient took home a complete set of cones and starting with the passive weight was instructed to retain the cone for 15 min twice a day. Once successful on two consecutive occasions, she proceeded to use the next heaviest weight.

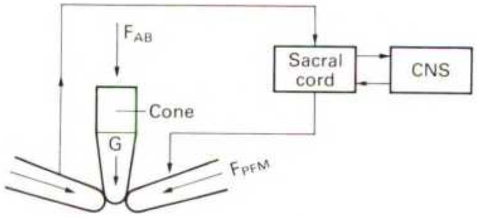

Fig. 2. Diagrammatic representation of forces on the cones when *in situ*. G, Weight of the cone; F_{AB}, force due to abdominal presssure; F_{PFM}, force developed by pelvic floor muscles.

Assessment after training

At the end of 1 month of use, the patient was reassessed in the same manner as before treatment. Patients by this time were aware of which muscles they should voluntarily contract, and those who noted subjective improvement continued their pelvic floor exercises at home. They were instructed to repeat the active cone exercise and to tense their pelvic floor muscles for 5 s, then release, five times in succession. They repeated this exercise at least 10 times daily and after 1 month they were asked to report their subjective impression of whether the previous improvement had been maintained.

Methods, definitions and units conform to the standards recommended by the International Continence Society.

Statistical analysis

Spearman's rank correlation and Mann–Whitney *U*-tests were used for comparison of decreased urine loss versus increased retained cone weight and subjective improvement versus increased retained cone weight. Wilcoxon matched-pairs signed rank test was used to compare pre- and post-treatment values.

Results

Thirty patients completed 1 month of cone training and 70% reported cure or improvement. Of the 30 women 19 (63%) continued pelvic floor exercises after 1 month and 11 (37%) opted for surgery. Nine patients showed no improvement in urine loss with therapy; seven of these chose surgery but two patients felt some benefit from pelvic floor exercises and continued with them (Fig. 3).

Table 1 details the urine loss, passive and active cone weights before and after exercise treatment, and Table 2 shows the statistical tests applied. A highly significant difference was found in the ability to retain heavier weights after treatment between the improved group and the failed group. The change in pad weight was correlated with the change in cone weights using Spearman's rank correlation and showed a highly significant correlation ($P<0{\cdot}001$, $r_s = -0{\cdot}827$ and $-0{\cdot}21$) between decreased urine loss and increased retained cone weight. Subjective improvement compared well with increased passive cone weight ($P = 0{\cdot}016$, Mann–Whitney *U*-test) but not with active cone weight. Of the 30 patients 27 found this an acceptable method of treatment. Of the 19 patients who continued exercises at home for a further month 13 (68%) had maintained their earlier subjective improvement, 4 (21%) had regressed but were still happy with the situation, and 2 (11%) were incontinent and unhappy. Those who regressed or became incontinent admitted that it was probably because they had not been conscientious about their pelvic floor exercises.

Discussion

Cone therapy is successful in reducing urinary loss due to genuine stress incontinence. The conventional management of genuine stress incontinence is a choice between surgery or conservative methods such as drug treatment, electrical stimulation, mechanical aids or physiotherapy. Cone therapy is less invasive than surgery, can be performed by the patient at home without disrupting home life, is considerably less expensive and may be as effective as surgical correction.

Cone therapy offers an easy method of distinguishing the use of pelvic floor muscles from abdominal wall contraction, as raised intra-abdominal pressure will enhance the downward force of the cone on the pelvic floor, increasing the exercise effect. Physiotherapists will sometimes employ a Kegel-type perineometer to teach the patient which muscles to contract but this can be deceptive as both abdominal wall and pelvic floor contraction will raise the level registered.

There was a 90% acceptability of the method

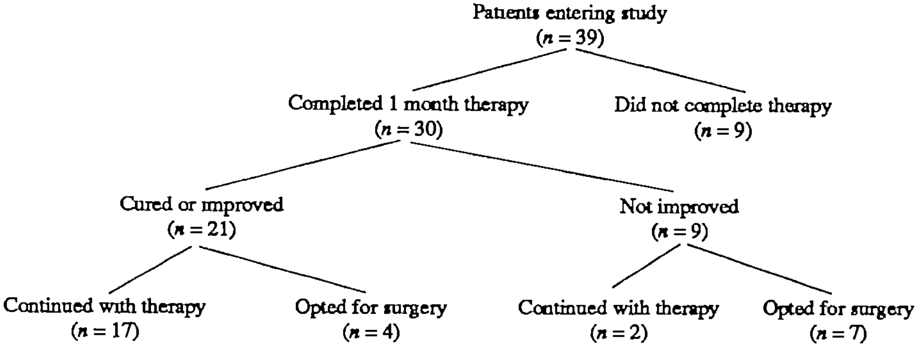

Fig. 3. Outcome of treatment with weighted vaginal cones in 39 patients with genuine urinary stress incontinence.

Table 1. Urine loss and passive and active cone weights before and after 1 month of training (Wilcoxon matched-pairs signed rank test)

Patient	Urine loss (g)		Passive cone weight (g)		Active cone weight (g)		Outcome	
	Before	After	Before	After	Before	After	Subjective	Surgery
1	13·2	5·1	20	50	20	60	I	PFE
2	2·0	1·3	20	40	30	40	I	S
3	58·1	18·2	40	50	50	50	I	S
4	6·1	0·2	20	50	30	60	I	PFE
5	24·0	20·1	0	20	0	20	N	S
6	4·4	0·1	70	60	80	70	I	PFE
7	31·4	44·9	20	20	30	30	N	S
8	35·9	11·4	40	50	50	50	I	PFE
9	11·5	79·4	60	50	70	60	N	S
10	25·4	36·1	40	30	50	30	N	PFE
11	0·4	7·4	30	50	40	60	N	PFE
12	5·1	6.4	30	40	40	50	I	S
13	40·1	32·9	40	30	60	30	N	S
14	4·6	0·2	30	60	30	70	I	PFE
15	17·7	0·0	30	40	30	40	I	PFE
16	21·1	5·1	50	70	60	70	I	PFE
17	56·5	5·3	20	50	30	50	I	PFE
18	29·9	43·7	20	<20	20	30	N	S
19	8·1	0·0	60	80	70	80	I	PFE
20	31·6	5·6	30	70	40	80	I	PFE
21	17·7	0·6	40	100	50	100	I	PFE
22	18·0	3·5	30	20	40	30	I	PFE
23	28·4	0·0	30	40	40	60	I	PFE
24	30·6	10·4	30	40	30	40	I	PFE
25	29·8	65·7	20	40	20	50	N	S
26	21·2	3·5	20	20	30	20	I	PFE
27	27·3	9·3	40	50	40	60	I	PFE
28	3·0	2·6	30	50	50	60	I	PFE
29	58·9	39·1	30	50	30	50	I	S
30	21·6	41·8	20	20	20	20	N	S
Median change in 1 month	−8·1 g		+10 g		+10 g			
Significance	P=0·033		P=0·0013		P=0·009			

I, Cured/improved; N not improved; S, proceeded to surgery; PFE, continued pelvic floor exercises.

Table 2. Statistical comparison of altered cone weights with subjective improvement and changed urine loss

Variable	Statistical test	Result	
Δ Passive cones *vs* subjective improvement	Mann–Whitney U	U = 146·5	P = 0·016
Δ Active cones *vs* subjective improvement	Mann–Whitney U	U = 126·5	P = 0·14
Δ Passive cones *vs* Δ pad weight	Spearman's rank correlation coefficient	r_s = −0·827	P<0·001 (two-tailed)
Δ Active cones *vs* Δ pad weight	Spearman's rank correlation coefficient	r_s = −0·21	P<0·001 (two-tailed)

in our premenopausal patients and 63% (19) of patients continued pelvic floor exercises at home after the month of cone training. This is important as it is essential for exercises to be maintained regularly for a beneficial effect to be seen. The set of cones can easily be used at home by the patient. After 1 month, patients have an awareness of the pelvic floor muscles and no longer require cones to perform their exercises. It is hoped they incorporate their exercises into their daily routine, as obviously exercises must be continued lifelong to maintain the benefit.

The nine patients who did not complete the therapy included one patient who became pregnant, one who developed a back complaint, one whose menorrhagia prevented regular use of cones, one who underwent surgery at another centre whilst using cones and five who were poorly compliant. Each of these five patients cited social circumstances as the reason for noncompliance rather than dislike of the method.

The decision to study premenopausal patients was based on the observations that ageing of skeletal muscle is associated with reduction in speed, strength and duration of muscle contraction (Carlson 1949). Work on external stimulating devices for the treatment of urinary incontinence found that the success rate fell as age increased (Doyle *et al.* 1974). In well-motivated postmenopausal patients, a beneficial effect of cone therapy may be possible. However, vaginal atrophy may also limit use of the weighted cones.

Our results show a significant correlation between the patients' appreciation of improvement and a heavier cone weight held passively, which we have assumed to represent the resting tone of the pelvic floor muscles. In nine patients there was no improvement in passive cone weight retained which correlated with those patients who felt no benefit from exercises. This group may not have been performing their exercises on a regular basis.

The objective results showed a highly significant correlation between ability to hold heavier cones and a reduction in urine loss. This suggests that it may be reasonable to offer all patients with genuine stress incontinence cone training whilst awaiting corrective surgery. If they improve and are happy with the result, surgery, with its recognized complication and failure rate, may be avoided.

This innovative approach to pelvic floor exercises is an advance in our management of genuine stress incontinence in the premenopausal woman.

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Tape measurement of symphysis–fundal height in twin pregnancies

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Summary. We hypothesized that tape measurement of symphysis–fundal height could be useful in detecting multiple pregnancies, and in such cases in predicting preterm labour and identifying small-for-gestational-age (SGA) fetuses. Although mean fundal height is substantially greater in twin than in singleton pregnancies, the value of the technique to detect multiple pregnancies may be limited because combined birthweight is less and the frequency of SGA fetuses is greater in undiagnosed than in diagnosed twin pregnancies. We found no evidence that the measured fundal height is greater in pregnancies going on to preterm delivery, and we suggest that the commonly accepted theory that the high rate of preterm labour in twin pregnancies results simply from uterine overdistension be reappraised. Nevertheless, fundal height measurement was of value in identifying pregnancies in which both babies were SGA and is therefore recommended as a simple, inexpensive and non-invasive technique.

Multiple pregnancy is associated with a high rate of perinatal loss due to preterm delivery (the most important cause) with further contributions from fetal growth retardation and malformation (Patel *et al.* 1984). The increased prevalence of preterm labour is generally attributed to uterine overdistension, it being thought that uterine contractions commence at a critical degree of myometrial stretch and then increase in frequency and strength to cause, ultimately, progressive dilatation of the cervix in labour (Quilligan 1981).

Twins that are small-for-gestational-age (SGA) at birth are also common (Manlan & Scott 1978) and are difficult to identify antenatally. Estimation of fetal size by abdominal palpation is made more difficult by the presence of the other twin, and biochemical investigation is of little value because only the combined endocrine output from both fetoplacental units can be assessed (Westergaard *et al.* 1985). Complex ultrasound measurements of twin fetuses may be highly sensitive in predicting SGA babies (Neilson 1981), and early reports of Doppler ultrasound indicate potential usefulness (Farmakides *et al.* 1985; Giles *et al.* 1985; Nimrod *et al.* 1987) but these techniques are not yet widely available. Tape measurement of symphysis–fundal height is becoming a widely used method to identify the SGA fetus in singleton pregnancies with reported sensitivities ranging between 55% (Rosenberg *et al.* 1982) and 89% (Belizan *et al.* 1978). Pearce & Campbell (1987) compared measurements of fundal height with ultrasound estimation of fetal abdominal circumference as screening tests for identifying SGA singleton fetuses; when the specificities of both techniques

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were adjusted to match at 79%, their sensitivities (76% and 83% respectively) were not greatly different.

Tape measurement of symphysis–fundal height is simple, inexpensive and non-invasive and is thus especially suitable for use in developing countries. In the management of multiple pregnancies, tape measurement could potentially be of value to aid diagnosis of multiple gestation, to predict preterm labour and to identify SGA fetuses.

Patients and methods

The Government and University obstetric services in Harare jointly supervise more than 40 000 deliveries a year, half in 13 community-sited clinics staffed by midwives and half (all high-risk pregnancies) in the central unit at Harare Central Hospital. Multiple pregnancy is regarded as of high risk and, if detected before or during labour, care is transferred to the central unit. A special antenatal 'twin clinic' was started at the hospital during September 1983, and all twin deliveries between October 1983 and April 1984 (numbering 515 pairs) were recorded. Of these patients 255 (50%) had attended the twin clinic (mean number of attendances 3·8; range 1–11), 161 (31%) had attended either a hospital or a community antenatal clinic, and 99 (19%) had had no antenatal care. In 194 pregnancies (booked or unbooked) the diagnosis of twins was not made before labour, and in 115 of these the first twin was delivered before the diagnosis was made.

At each attendance at the twin clinic (but at no other clinic) symphysis–fundal height was measured by one of two of us (J.P.N. or D.A.A.V.). The highest point of the uterus was identified, whether or not in the midline, and the tape measure held there using light pressure. The distance to the upper border of the pubic symphysis was then measured in centimetres by holding the tape taut with the marked surface downwards. Latterly, tape measurement of abdominal girth, performed with the patient supine, and at the level of her umbilicus, also became routine.

Gestational age at delivery was determined in twin clinic patients using a hierarchy of assessments: ultrasound measurement before 24 weeks (12 pregnancies), Dubowitz scoring (213), ultrasound examination between 24 and 30 weeks (9) and last menstrual period alone (16); five patients had unknown dates and were excluded from the analyses. In the 'Dubowitz group', 47 patients (22%) had unknown dates and in a further 30 pregnancies (14%), there was a difference of more than 2 weeks between gestational age calculated from the last menstrual period and that based on neonatal examination; in the majority (64%), menstrual history and Dubowitz scoring, which has been validated in African populations (Brueton *et al.* 1973; Singer *et al.* 1973; Raghu *et al.* 1981) were compatible. Delivery before 37 completed weeks was classified as preterm, and before 34 weeks as 'early preterm'. Tocolytic drugs were not used.

Birthweight-for-gestational-age charts for this population have recently been compiled from 5571 singleton livebirths occurring at or later than 32 weeks of pregnancy (S. P. Munjanja, unpublished data). Twin babies, excepting those born before 32 weeks, with birthweights on or below the 5th centile on this chart were classified as SGA. In 10 pregnancies one or both babies were stillborn and macerated and were not included in our analyses (below).

Statistical analyses utilized Student's t-test or χ^2-tests as appropriate.

Results

Among patients who had any type of antenatal care, those whose twin pregnancies were diagnosed had greater combined birthweights with a mean value of 4·80 kg (SD 0·95) compared with 4·05 kg (SD 1·01) for the undiagnosed twins ($t = 6{\cdot}47$, $P<0{\cdot}001$); gestational age at delivery was also greater: 36·0 weeks (SD 2·9) compared with 35·0 (SD 2·6; $t = 2{\cdot}99$, $P<0{\cdot}003$). The combined birthweights were significantly different between these two groups at the 5% level at 33 to 35 weeks, but not thereafter. There were no significant differences between these groups in maternal age, height or weight or in gestational age at first clinic visit. The distribution of SGA babies with time of diagnosis of twin pregnancy is shown in Table 1.

To assess whether fundal height measurement might have helped predict preterm labour, we considered only those patients who had attended the twin clinic and were delivered after 28 weeks following spontaneous labour, thus excluding nine pregnancies in which induction of labour or elective caesarean section was performed. The study group numbered 236 and 115 (49%) of them were delivered preterm. Figure 1 com-

Table 1. Distribution of small-for-gestational-age (SGA) twins and time of diagnosis of twin pregnancy (percentage in parentheses)

		Diagnosis of twins			
		Antepartum		Intrapartum	
	n	*n*	(%)	*n*	(%)
Both twins SGA	41	25	(61)	16	(39)
One twin SGA	98	74	(76)	24	(24)
Neither twin SGA	234	193	(82)	41	(18)

χ^2 10·091, $P = 0·006$.

pares the fundal height measurements by gestation between the women delivered at term and those delivered preterm; the women delivered at term had a greater mean height although the difference was statistically significant at the 5% level only at 27 and 30 weeks. The same pattern was found when fundal height measurements were compared between those delivered early preterm (<34 weeks) and those delivered at term, and when abdominal girth measurements were compared, instead of fundal heights.

To assess the value of fundal height measurement in identifying SGA fetuses, measurements obtained from only those 231 women who were delivered at or after 32 weeks were analysed. In 19 (8%) of these both twins were SGA, in 52 (22%) only one twin was SGA, and in the remaining 160 (67%) neither twin was SGA. Mean fundal height measurements were lower at each week in those women who gave birth to two SGA babies compared with those who gave birth to two appropriate-for-gestational-age (AGA) babies (Fig. 2); there was a less striking difference in the measurements from those pregnancies in which only one fetus was SGA. Centile values for fundal height measurements from pregnancies resulting only in AGA babies were obtained for each week of gestation between 28 and 39 weeks using the Statistical Package for the Social Sciences (SPSSX) and smoothed using the Minitab package. The smoothed 10th centile thus obtained was evaluated as a means of separating the different categories of pregnancy outcome (Table 2) and the longitudinal results from the 19 pregnancies producing two SGA babies are shown superimposed on the normal range in Fig. 3.

Discussion

We hypothesized that tape measurement of symphysis–fundal height might contribute to the management of multiple pregnancies by aiding diagnosis, predicting preterm labour and identifying SGA fetuses. Because it was not a routine

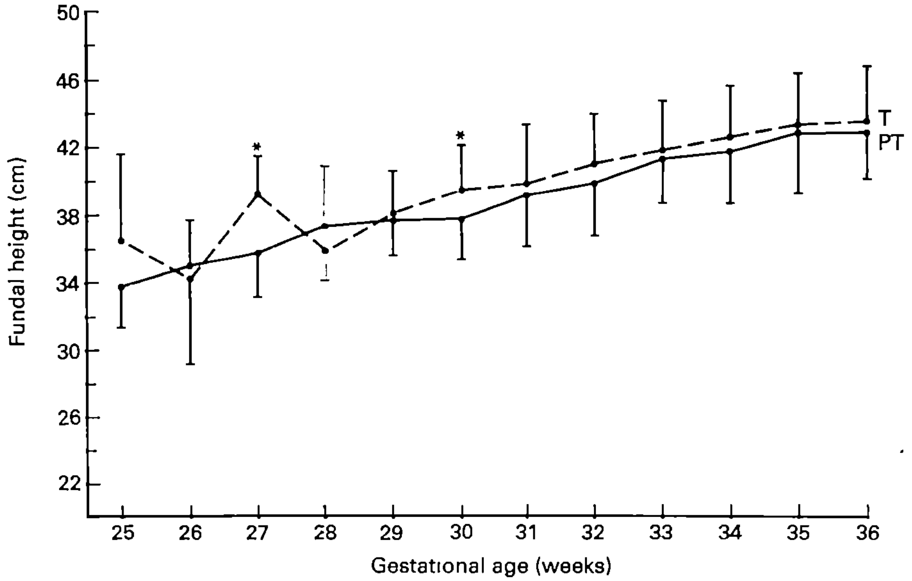

Fig. 1. Mean symphysis–fundal height by gestation in twin pregnancies. – – – – T, 121 women delivered at term; ——— PT, 115 women delivered preterm. Vertical bars represent the standard deviation. *$P<0·05$.

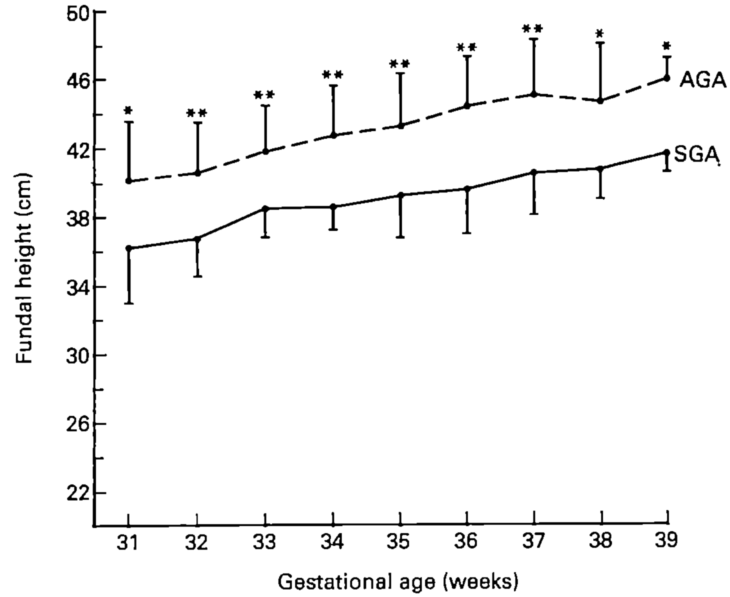

Fig. 2. Mean symphysis–fundal height by gestation in twin pregnancies. – – – – AGA, 160 women who gave birth to two appropriate-for-gestational-age babies; ——— SGA, 19 women who gave birth to two small-for-gestational-age babies. Vertical bars represent the standard deviation. *$P<0{\cdot}05$; **$P<0{\cdot}01$.

at any other of the hospital's clinics we do not have comparable data from singleton pregnancies in this population or from those twin pregnancies which went undiagnosed during antenatal care. However, mean fundal heights in this study were substantially greater at each week than those reported in singleton pregnancies (Calvert *et al.* 1982) confirming that they may help identify mulitple pregnancies during antenatal care. On the other hand, our findings that combined birthweight tends to be lower and the prevalence of SGA babies greater in undiagnosed than in diagnosed twin pregnancies suggests that it is precisely because the uterus (and therefore the fundal height) is not unusually large that the diagnosis may not be established in these pregnancies. This requires prospective study but is a matter of critical importance in a situation, as in Harare, where facilities available for birth vary greatly (between hospital and community clinic) and because of the hazards of twin births (especially when unexpected).

Table 2. Tape measurements of symphysis–fundal height and prediction of outcome

Gestation (weeks)	No. of measurements	Sensitivity (%) SGA×2	SGA/AGA	Specificity (%) AGA×2
32	90	67	29	87
33	101	40	22	91
34	116	56	22	91
35	123	60	15	90
36	106	63	21	89
37	75	25	20	93
38	42	75	29	92

Sensitivity has been calculated for alternative abnormal outcomes of both babies small-for-gestational-age (SGA×2) and only one SGA baby (SGA/AGA). Specificity is the percentage of pregnancies in which both babies are appropriate-for-gestational-age (AGA×2) which would have been identified as having normal fundal height measurements.

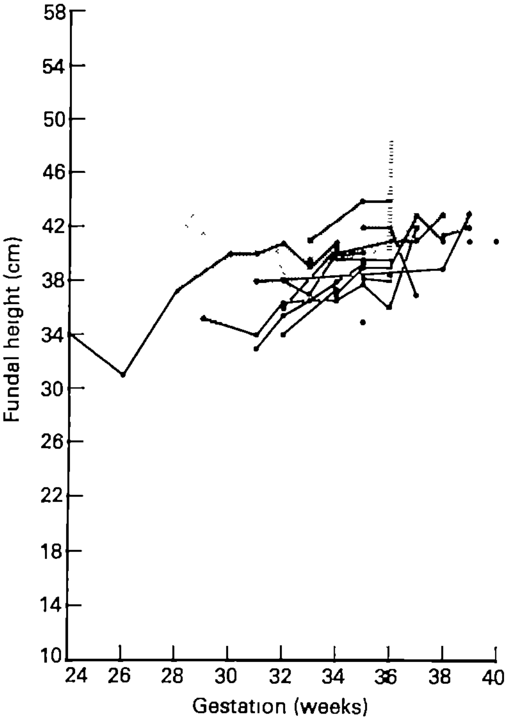

Fig. 3. Symphysis–fundal height measurements by gestation in 19 twin pregnancies resulting in both babies small-for-gestational-age. The shaded area represents the normal range.

Because the high rate of preterm birth in twin pregnancies (49% in our series) is generally attributed to uterine overdistension, we hypothesized that preterm labour might be predicted by high fundal height measurements. We found the reverse, i.e. that mean fundal height (and abdominal girth) tended to be lower in women who were delivered preterm. Although tape measurement of these variables is a crude way to assess uterine size, our findings are consistent with those of a small study in which more precise measurements (of total intrauterine volume) were made with ultrasound (Redford 1982). Acute increases in intrauterine volume may induce uterine activity (Csapo *et al.* 1963) and may not be identified by examinations separated by a week. However, such acute changes could only occur by rapid accumulation of amniotic fluid and we found polyhydramnios identified during labour or at the twin clinic to be extremely rare. The overdistension theory does not explain intragroup differences and needs reappraisal.

The use of singleton standards to classify SGA twins is controversial, but there is a strong argument for doing so (Neilson 1981). Using the 5th centile obtained from locally studied singleton births, 30% of our twin clinic pregnancies resulted in either one or two SGA babies. Tape measurement of symphysis–fundal height would have proved useful in identifying those pregnancies in which both babies were SGA although these were relatively few, thus limiting the predictive value. The technique would not have been useful in the minority of pregnancies in which gestational age was uncertain before delivery. The calculated sensitivities were not derived from identical groups of patients at each week of gestation and therefore no conclusions about the optimal time of assessment may be drawn. Fundal height measurement did not prove useful when only one of twin babies was SGA and this highlights the need for further study of techniques (e.g. ultrasound) which assess each twin fetus. Despite these reservations, we recommend tape measurement of symphysis–fundal height as a simple, non-invasive and inexpensive technique, for the routine antenatal assessment of twin, as well as singleton, pregnancies.

Acknowledgments

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Closed suction wound drainage and lower-segment caesarean section

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Summary. In a randomized controlled study of wound suction drainage after transverse suprapubic incision for lower-segment caesarean section no significant advantages could be demonstrated for routine drainage in terms of wound infection, haematoma formation, duration of hospital stay or analgesic requirements.

The transverse suprapubic incision is often used for lower-segment caesarean section. The advantages of this approach include a more satisfactory cosmetic result for the patient, and a substantial reduction in the frequency of postoperative wound dehiscence compared with vertical incisions (Mowat & Bonnar 1971).

With a vertical midline wound all tissues are incised in the same plane, but with a transverse suprapubic approach the skin, fat and rectus sheath are incised transversely whilst the rectus abdominis muscles are separated longitudinally in the midline. This produces a large potential space between the rectus sheath and rectus muscles, and in the process many perforating blood vessels passing from muscle to fascia may be disrupted producing haemorrhage in this area. For this reason many obstetricians drain this space as a routine, believing that the frequency of wound haematoma and infection will thereby be reduced. This randomized controlled study was designed to ascertain whether routine suction drainage of these wounds at the time of caesarean section produced any obvious clinical benefit.

Patients and methods

At the Northern General Hospital, Sheffield, patients who were delivered by a lower-segment caesarean section through a transverse abdominal wound were recruited to the study, hospital ethics committee approval having been obtained. Most operations were performed by junior staff in the registrar grade. After closure of the uterus, abdominal closure was with a standard technique. Randomization used 200 sealed envelopes containing the instructions on drainage or non-drainage (100 in each category) which were shuffled thoroughly and stored in the obstetric theatre. Surgeons were given the option to exclude patients from the study if they felt that bleeding from the wound was of a degree sufficient to warrant elective drainage. However, such exclusions were discouraged and once it became obvious that undrained wounds were not associated with undue morbidity the practice of elective drainage virtually ceased. Before the rectus sheath was closed, haemostasis was secured with coagulation diathermy, and then a sealed envelope was opened containing the instruction to drain the wound or not. The Redivac (Athrodax Surgical Ltd, Bicester) drainage system uses a pre-exhausted drainage bottle with appropriate connecting tubing and a fine-guage perforated drain with a lumen 2 mm in diameter. Drains were placed behind the rectus sheath and brought out of the abdomen through a stab incision lateral to the wound. The rectus sheath was then sutured with continuous 'O' vicryl (Polyglactin 910, Ethicon Ltd), and skin closure was with interrupted 'O' silk. The drains were removed after 24 h unless drainage was still occurring. Wound assessment was undertaken using the scoring method described by Wilson *et al.* (1986). During the postoperative

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Table 1. Points scale for wound inspection*

	Proportion of wound affected (%)					
Wound characteristic	0	<20	20–39	40–59	60–79	>80
Serous exudate	0	1	2	3	4	5
Erythema	0	1	2	3	4	5
Purulent exudate	0	2	4	6	8	10
Separation of deep tissues	0	2	4	6	8	10

* From Wilson *et al.* (1986); 10 points are added for isolation of bacteria, 10 points for antibiotics and 5 points for prolonged stay; category of infection defined by total score: 0–10, satisfactory; 11–20, disturbance in healing; 21–30, minor wound infection; 31–40, moderate wound infection; >40, severe wound infection.

Table 2. Characteristics of the two groups of women delivered by caesarean section

Variable	Drain (n=100)	No drain (n=100)
Number of elective procedures	38	37
Mean maternal weight (kg) (SD)	76·3 (11·9)	73·4 (12·3)
Mean wound score (range)	2·34 (0–36)	2·33 (0–33)
Minor wound infection (21–30 points)	1	2
Moderate wound infection (31–40 points)	4	3
Haematoma	1	0
Bruising	3	3
Postoperative pyrexia	28	26
Mean white cell count (day 3) (SD)	13·3 (4·4)	12·9 (3·1)
Mean hospital stay (days) (SD)	6·06 (1·27)	6·16 (1·0)
Mean opiate doses administered (SD)	4·03 (2·3)	4·06 (1·8)
Mean anti-inflammatory analgesic dosage (SD)	5·6 (4·3)	5·8 (4·3)
Mean drainage volume (ml)	53 (0–300)	—

There were no statistically significant differences between the two groups using Student's *t*-test or χ^2-test with Yates' correction as appropriate.

period evidence of serous or purulent exudate, erythema, or separation of wound edges was sought and if present an estimate was made of the proportion of the wound so affected. Wounds were assessed on the second, fourth and sixth days of the puerperium and using the point scale (Table 1) a cumulative score was calculated. Further points were added to the total if bacteria were isolated from the wound, antibiotics were required for wound infection or the patient had a prolonged postoperative stay (>7 days).

Total scores obtained in this manner should reflect the severity of any wound problems, and we found a good correlation between the scoring system and clinical evidence of wound infection.

In addition, hospital stay, analgesic requirements, and wound haematoma formation were recorded. Statistical analysis was with a 'BSTAT' computer package.

Results

A total of 200 patients was entered into the study (100 patients underwent drainage of the wound and 100 did not). There were no significant differences between the two groups (Table 2).

The proportion of elective and emergency procedures was the same in each group. No difference in outcome was seen between the groups in those patients having their second or third caesarean section through the same incision.

Discussion

Many obstetricians routinely employ suction drainage of transverse suprapubic wounds at the time of caesarean section although one large questionnaire study of wound infection after caesarean section failed to demonstrate any major difference in the frequency of infection between patients who were drained and those who were not drained (8·8% *vs* 5·3%) (Moir-Bussy *et al.* 1984).

In other branches of surgery closed suction drainage has been associated with lower wound infection rates and shorter hospital in-patient stay (Maitland & Mathieson 1970; Morris 1973). After radical mastectomy, Morris found wound breakdown to be less common and the average duration of hospital stay to be 7 days less where suction drainage had been employed. We have been unable to demonstrate any such differences with the transverse suprapubic wound but then it is a much less extensive dissection and is associated with only a fraction of the volume of drainage occurring after radical mastectomy. In addition, the blood supply of the flaps of the mastectomy wound is much more tenuous than that of the lower abdominal wound and it is easy to envisage that the presence of a large collection of fluid under these flaps would encourage wound breakdown.

The present study employed a version of the wound scoring system of Wilson *et al.* (1986) as this appears to be a sensitive method of detecting minor degrees of abnormal wound healing. Differences in treatment groups may thus be highlighted more readily than when merely recording the occurrence of major wound sepsis, and the power of any study is thereby increased. Even using this scoring system no difference in outcome was demonstrable between the two groups of patients. It is of interest that the only patient to develop a clinically obvious wound haematoma had had a wound drain inserted. Minor differences in the occurrence of wound complications may not be identified unless a prospective study is undertaken containing several 1000 patients, but the information available from this and other studies would seem to indicate that the extra time and expense involved in using suction drainage routinely for transverse suprapubic wounds at the time of caesarean section does not produce any obvious clinical benefit. It must be stressed, however, that our study was of routine drainage and we would still recommend the insertion of a drain if good haemostasis could not be secured because of surgical or haematological problems.

Acknowledgments

We would like to thank the medical and midwifery staff at the Northern General Hospital for their co-operation in the running of this study. Thanks to V. Aram for secretarial assistance.

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Surgical wound drainage: a survey of practices among gynaecologists in the British Isles

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Summary. All 2836 members and fellows of the Royal College of Obstetricians and Gynaecologists were circulated with a questionnaire concerning their practices with regard to wound drainage. The overall response rate was 43%, although that from practitioners of consultant and senior registrar status was 67%. The use of wound drainage was consistent between surgeons of differing levels of experience and different subspecialty interests within gynaecology. At routine 'clean' operations the use of drains is limited; only 0·4% of gynaecologists drain the peritoneal cavity, 1% the pelvis, 4% the subcutaneous tissues, and 20% the rectus sheath routinely. At more specialist 'clean' procedures, however, greater use of drains is made; at suprapubic incontinence operations 51% of surgeons drain the retropubic space; at radical hysterectomy 55% drain the pelvis; and at radical vulvectomy 63% use drains in the groins, routinely. In all the above operations much greater use is made of active (83%) than passive drains (17%). With potentially contaminated wounds, however, 46% of gynaecologists use a passive drain.

It was originally stated by Halstead (1898) and often repeated since (Cruse & Foord 1973; Agrama *et al.* 1976; Magee *et al.* 1976; Moss 1981; Helmkamp, Krebs & Amstey 1984) that drains must not be considered a substitute for haemostasis nor a replacement for meticulous surgical technique. The place of, and most appropriate techniques for, drainage in surgical practice have always been the source of contention. Price (1888) put forward the view, perhaps not unrepresentative of current practices: 'There are those who ardently advocate it—there are those who in great part reject it—there are those who, Laodicean-like, are lukewarm concerning it, and finally, some who, without convictions, are either for or against it, use it or dispense with it, as chance or whim, not logic may determine'. Drains are used therapeutically in the presence of purulent material, necrotic debris, a fistula or to prevent premature closure of a wound. The prophylactic use of drains is intended to prevent the accumulation of blood, lymph, urine, pus, intestinal contents, bile or pancreatic secretions, and occasionally to permit the early detection of surgical complications. It is in this latter context that the use of drains is perhaps most controversial (Helmkamp *et al.* 1984), and it is here that the gynaecologist is most frequently concerned. Whilst there is a considerable surgical literature regarding the different techniques of wound drainage, there is little information concerning actual practices among gynaecologists. The aim of this study was to establish current practices with respect to wound drainage among gynaecologists in the British Isles.

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Methods

All members and fellows registered with the Royal College of Obstetricians and Gynaecologists (RCOG) at the end of 1985 and resident within the British Isles were circulated with a two-part reply-paid questionnaire relating to their use of catheters and surgical drains; results of the catheter survey form the basis of a separate publication (Hilton 1988). In the second part of the questionnaire respondents were requested to indicate whether they would routinely, selectively, or never use drains in the pelvis, peritoneal cavity, rectus sheath, or subcutaneous tissues at a variety of obstetric and gynaecological procedures; in those situations in which drainage was advocated they were asked to state what type of drain was used, and in particular to indicate whether a suction or passive drain, and an open or closed system, were preferred. In an effort to assess regional variations in practice and the influence of experience and specialist interests within gynaecology, respondents were also asked to state the time since they had obtained membership of the RCOG, the region in which they were currently employed, whether they had a major specialist interest in gynaecological oncology or urology, and the extent of their surgical workload in general gynaecology or in these specialist areas.

Results

Respondents

A total of 2836 questionnaires was distributed and 1216 were returned, giving an overall response rate of 43%. Of the returns, 269 were uncompleted: 153 from retired members or fellows, 84 from members practising in specialties other than obstetrics and gynaecology, 29 were returned by the Post Office undelivered, and three were returned by relatives of deceased members or fellows; 947 completed questionnaires were returned by members and fellows currently active within the specialty—'the appropriate respondents'—(78% of returns; 33% of the circulation). Of the 947 appropriate respondents 683 (72%) were of consultant status, 130 (14%) of senior registrar status, and 115 (12%) were registrars; the remaining 19 (2%) were made up of hospital practitioners, clinical assistants, senior house officers or of unspecified status. Based on predicted figures for 1986 from the Report of the Manpower Advisory Sub-Committee of the RCOG (1983) the response rate from consultants was approximately 65% and from senior registrars 78%.

Of the 947 appropriate respondents 183 (19%) considered themselves to have a major specialist interest in gynaecological urology; in subsequent analysis the practices of the other 764 generalist surgeons were compared with those of the 183 who claimed a major specialist interest in gynaecologic urology, and with practices of 47 surgeons who had a significant surgical workload in the subspecialty—arbitrarily defined for these purposes as more than 25 suprapubic incontinence procedures per year.

A total of 178 (19%) of the appropriate respondents considered themselves to have a major specialist interest in gynaecological oncology; again, the practices of the other 769 surgeons with no particular interest in gynaecological oncology were compared with the practices of those claiming a major specialist interest and with the practices of 56 respondents who had a significant surgical workload in the subspecialty, defined for these purposes as more than 25 radical cancer procedures per year.

The use of drains

The number of surgeons employing drains on a routine or selective basis at a variety of obstetric and gynaecological procedures is shown in Table 1 and Fig. 1. At what one might call routine 'clean' operations (caesarean section, abdominal hysterectomy, and ovarian cystectomy) the use of drains on a routine basis is limited; averaging for these procedures, only 0·4% of gynaecologists drain the peritoneal cavity routinely, 1% drain the pelvis, 4% the subcutaneous tissues, and 19% the rectus sheath. With potentially contaminated wounds, following appendicectomy or other bowel surgery, the routine use of drains is similarly limited, although at more specialist 'clean' procedures, particularly where large parietal spaces are dissected, greater use of drains is made. At suprapubic incontinence operations 50·7% of surgeons drain the pelvis or rectus sheath (both presumably implying the retropubic space); at radical hysterectomy 55·5% drain the pelvis; and at radical vulvectomy 63·5% use drains in the groins.

Table 1. The use of drains by gynaecologists in the British Isles

	Percentage of respondents using drains routinely (selective use shown in parentheses)			
Surgical procedure	Pelvis	Peritoneum	Rectus sheath	Subcutaneous
Caesarean section				
Transverse incision	0·3	0·0	22·8	4·4
	(14·3)	(11·9)	(45·5)	(22·2)
Longitudinal incision	0·4	0·2	4·9	1·7
	(14·1)	(12·2)	(27·0)	(17·7)
Abdominal hysterectomy	3·1	1·4	19·8	4·2
	(57·5)	(29·1)	(47·5)	(24·8)
Ovarian cystectomy	0·2	0·2	15·9	3·5
	(18·8)	(15·5)	(37·4)	(18·2)
Appendicectomy	1·5	1·0	30·4	0·9
	(31·7)	(40·4)	(19·6)	(13·4)
Other bowel surgery	6·8	6·3	6·7	2·1
	(50·8)	(48·9)	(33·8)	(19·4)
Suprapubic incontinence operation	15·9	6·8	34·7	5·4
	(13·5)	(6·9)	(26·4)	(17·3)
Radical hysterectomy	55·5	13·8	22·4	3·7
	(28·0)	(28·1)	(39·6)	(20·9)
Radical vulvectomy	8·8	1·1	7·4	63·5
	(3·8)	(2·9)	(4·1)	(12·5)

The figures quoted for each operation are the percentage of gynaecologists using drains routinely in the sites listed; figures shown in parentheses are those using drains in selected cases only.

Drain types

In all the 'clean' procedures referred to above much greater use is made of active than passive drains (see Table 2); averaging for all these procedures, 17% of gynaecologists favour a passive type of drain, and 83% an active suction drain; of the latter, 81% use high-pressure and 12% low-pressure drainage systems (7% being unspecified). Following bowel surgery greater advocacy of passive drains is apparent. Here, of those gynaecologists using drains, 46% employ passive drains; where suction drains were used, however, the proportion of surgeons advocating high- and low-pressure drain systems was similar to those with 'clean' wounds.

The effect of specialist interest

The influence of a subspecialty interest in urology or oncology on surgeons' practices with regard to wound drainage is examined in Table 3. Although there is a tendency for those individuals with an interest in gynaecological urology to drain the retropubic space more frequently following suprapubic incontinence surgery, and for those with an interest in gynaecological oncology to drain the pelvis more frequently following radical hysterectomy, these differences are not statistically significant (χ^2-test).

Discussion

In a parallel study into practices with regard to bladder drainage amongst gynaecologists in the British Isles (Hilton 1988), considerable variations in practices were identified between surgeons of differing experience and different levels of interest in gynaecological subspecialties; regional variations were also seen. Although considerable variations in practices are evident in the present survey, as found in previous surveys among general surgeons (Smith & Gilmore 1985), the above associations were not recognized.

Closed wound suction was first employed by Raffl in 1952, and the use of the portable closed wound suction unit was introduced by Redon & Jost in 1954 (reviewed by Moss 1981). Such systems may be said to be almost synonymous with wound drainage in current surgical prac-

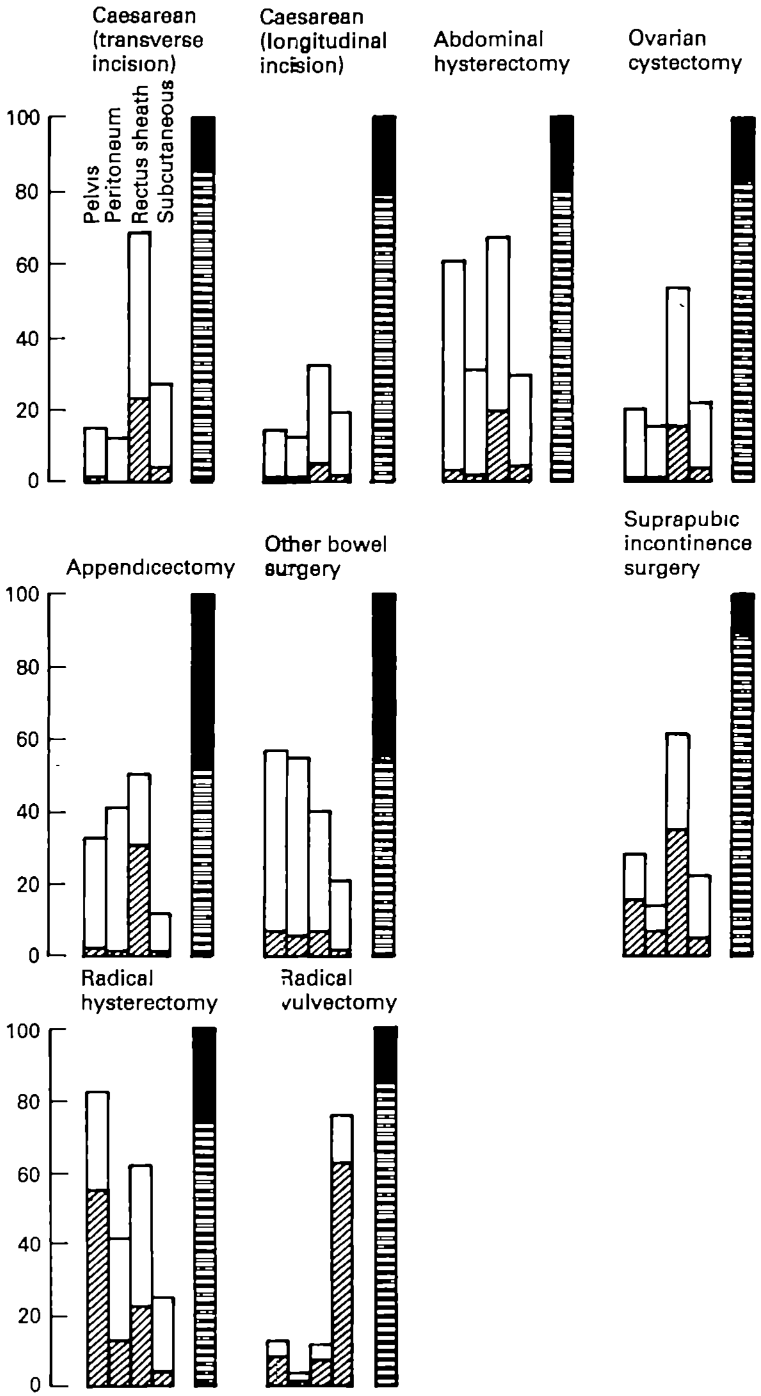

Fig. 1. Overall use of drains. For each operation the percentage of gynaecologists using drains routinely or in selected cases in the pelvis, peritoneal cavity, rectus sheath, or subcutaneous tissues is shown by the first four columns; the right-hand column shows the percentage using passive and active drainage systems at the appropriate procedure. ▨, Routine drainage; □, selective drainage; ▤, suction drain; ■, passive drain.

Table 2. The use of drains by gynaecologists in the British Isles

Surgical procedure	Percentage of respondents using		Specified suction drain pressure		
	Passive drains	Suction drains	High	Low	Unspecified
Caesarean section					
Transverse incision	14	86	82	11	7
Longitudinal incision	21	79	82	12	6
Abdominal hysterectomy	19	81	81	12	7
Ovarian cystectomy	17	83	81	13	6
Appendicectomy	48	52	77	14	9
Other bowel surgery	44	56	78	13	9
Suprapubic incontinence operation	11	89	81	11	8
Radical hysterectomy	24	76	78	14	8
Radical vulvectomy	15	85	80	12	8

The figures quoted are the percentage of clinicians using drains in the listed operations who employ passive and active drainage systems; to the right are shown the percentage of those employing active drains who specified high- or low-pressure suction.

tice, and this fact is emphasized by their consistent and widespread use in all the operations examined where drainage of some sort is used. These drains are typified by the original reusable 'Redivac' system, although many disposable portable suction devices are now available with either high (300–500 mmHg) or low pressure (100–150 mmHg). The question of optimum suction pressure was investigated by Britton *et al.* (1979); they found that low-pressure drains collected more fluid, and were required to stay in place longer than high-pressure systems, and required frequent recharging. Infection has been shown to spread retrogradely via low-pressure suction systems, and this occurs particularly at the time of recharging (Lumley *et al.* 1974); however, this may be obviated to some extent by the incorporation of a non-return valve as in some available systems (Seeley *et al.* 1979). High-pressure systems require little or no

Table 3. The effect of surgeon's level of interest in gynaecological urology or oncology on the use of routine wound drainage at suprapubic incontinence surgery, radical hysterectomy and radical vulvectomy respectively

Surgical procedure	Percentage of respondents using drains routinely			
	Pelvis	Peritoneum	Rectus sheath	Subcutaneous
Suprapubic incontinence surgery				
Generalist surgeons ($n = 866$)	16	7	35	5
Major urology interest ($n = 183$)	19	5	33	7
Major interest with >25 suprapubic operations/year ($n = 47$)	19	2	47	4
Radical hysterectomy				
Generalist surgeons ($n = 826$)	55	14	22	4
Major oncology interest ($n = 178$)	64	12	23	4
Major interest with >25 radical operations/year ($n = 56$)	65	9	24	5
Radical vulvectomy				
Generalist surgeons ($n = 821$)	9	1	7	63
Major oncology interest ($n = 169$)	11	0	7	74
Major interest with >25 radical operations/year ($n = 56$)	10	0	7	60

n, Number of responses, the disparities in numbers reflect different response rates to various questions.

recharging, and are therefore relatively free from this complication; their use at hysterectomy has been shown to reduce the frequency of febrile morbidities from 25% to 11% and from 32% to 8% at abdominal and vaginal operations respectively (Schwartz & Tanaree 1975, 1976). A significant disadvantage of high-pressure systems, however, is that tissue encroachment into the tubing holes may impair drainage and cause tissue damage; their use in the peritoneal cavity has been associated with bowel trauma leading to faeculent peritonitis (Benjamin 1980), internal hernia formation (Fulham & Pritchard 1985), and evisceration (Shoukris & Kiff 1984). Their use in therapeutic drainage of haematomata or frankly contaminated wounds is similarly undesirable since they are likely to obstruct more rapidly with purulent material or clot (Hilton 1987). It is thus perhaps somewhat surprising that of those surgeons advocating the use of routine drainage following bowel surgery, over half employ suction drains, and over three-quarters of those use high-pressure systems in this situation.

Based on a review of the literature, several recommendations relating to the use of drains in gynaecological practice have been made (Hilton 1987). Several of these recommendations are confirmed as the majority practice in this survey; they may be summarized as follows.

In abdominal and gynaecological surgery there is no evidence to support the routine use of peritoneal drains; however, where drains are used, a 'closed' passive system such as the Robinson drain or an 'open' active drain (sump drain) with a bacterial air inlet filter should be employed. Silicone is the preferred material since it generates the least tissue reaction.

For drainage of the parietes, e.g. the retropubic space at suprapubic incontinence surgery, or the groins at radical vulvectomy, closed wound suction drainage using high vacuum pressure is the most effective in obliterating 'dead space' and reducing the risks of infection.

Therapeutic drainage, of haematomata, frankly contaminated wounds, or abscess cavities is perhaps the only place in which an 'open' passive drain may be shown to be beneficial, but even here an 'open' active drain (sump drain) with an air inlet filter, or low-pressure 'closed suction drain' may again be used to advantage. High-pressure active drains are not recommended since they may obstruct more rapidly with purulent material or clot.

Where drains are left in proximity to blood vessels, nerves, bowel, or bladder, they should be of a soft material. If suction is to be applied it should be either vented 'open' or of low pressure, and tubing holes should be small.

Drains, whether active or passive, should never exit through the operative incision, if infection, dehiscence, and hernia formation are to be minimized.

Drains should be removed as soon as clinically significant drainage ceases. For prophylactic drains this will usually be within 24–48 h of operation, although may be much longer following lymphadenectomy. Drains placed prophylactically can be removed straight away; those used therapeutically should be advanced by 3–5 cm per day once drainage has ceased.

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Relapsing fever in pregnancy: analysis of high-risk factors

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Summary. The diagnosis of tick-borne relapsing fever was established in 27 pregnant patients by demonstration of *Borrelia* spirochaetes in a thick blood smear and the borrelia index was estimated to calculate the density of the spirochaetaemia. Clinical findings are described and compared with those reported from Rwanda. The results suggest that the density of the spirochaetaemia and the gestational age are the main high-risk factors.

Relapsing fever is an infectious febrile disease caused by *Borrelia,* a genus of bacteria in the family Spirochaetaceae; species designations are based on their respective arthropod vectors, ticks and lice. Tick-borne relapsing fever is transmitted by various soft ticks of the genus *Ornithodoros* (Goubau 1984), and in pregnant women it is often a clinically severe and dramatic infection.

In uncomplicated cases relapsing fever is characterized by episodes of fever only, separated by afebrile intervals (Bryceson 1976; Leithead 1971), but in patients with an intense spirochaetaemia (a high borrelia index) disorders of several organs and systems are present (Melkert 1987; Goubau 1984).

Tick-borne relapsing fever is an endemic disease in Sengerema district, Mwanza region, Tanzania (Melkert 1987), and the aim of this prospective study was to assess the relative importance of various factors, including age, gravidity, gestational age and intensity of the spirochaetaemia, in the severity of complications.

Patients and methods

Background

Sengerema Hospital is a designated district hospital, located in Mwanza region, Tanzania. It acts as a referral hospital for Sengerema district in which six health centres and 30 dispensaries are located. There are four regions in Tanzania where tick-borne relapsing fever is frequently diagnosed: Singida, Dodoma, Kigoma and Mwanza region (Melkert 1987).

The study included 27 pregnant patients, seen at Sengerema Hospital between September 1986 and March 1987, who had a positive blood film for *Borrelia.* Thick blood films were prepared from non-anticoagulated capillary blood. The slides were dried in air and stained with Giemsa diluted in buffered saline. The degree of spirochaetaemia was estimated by the borrelia index in all patients on admission by the author. The borrelia index is calculated from the number of spirochaetes counted against the number of white blood cells seen in one high-power field, similar to the reporting of parasite density in malaria (Cheesbrough 1981).

The type of antibiotic therapy given depended on the preference of the admitting doctor at the outpatient department. During their stay in hospital patients were seen daily so that any complications could be recognized.

Results

The 27 pregnant patients included in this study were between 15 and 42 years of age; 14 of them recovered well without any problem and seven showed one or more mild complications including: anaemia, mild jaundice without tender hepatomegaly and preterm delivery. The remaining six patients had one or more severe

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complications including: miscarriage, fetal death, pneumonia, hepatitis or severe jaundice with tender hepatomegaly and neonatal asphyxia.

There was no correlation between age distribution and the severity of complications (Table 1) nor between gravidity and complications (Table 2). Most of the complications were seen in early or late pregnancy, the women seen around 7 months gestation had only mild or no complications (Table 3).

Of the 11 patients with a mild spirochaetaemia (borrelia index 0·01–0·4), only one had a severe complication (miscarriage), the other 10 recovered well. Of the 10 patients with a moderate spirochaetaemia (borrelia index 0·5–1·9), five showed mild complications and one had a severe complication (intrauterine fetal death). All recovered well.

Of the six patients with a severe spirochaetaemia (borrelia index >2), two had mild complications and four had severe complications (hepatitis, neonatal asphyxia, pneumonia or intrauterine fetal death). Five of these women recovered but one died (Table 4).

Discussion

Tick-borne relapsing fever is widely distributed in disseminated foci around the world (Goubau 1984). It may present sporadically in some parts of the world (e.g. the USA), whereas in other places it is a highly endemic disease (e.g. Sengerema district, Tanzania, East Africa).

During pregnancy the borrelial infection is particularly intense, pregnant women show higher densities of spirochaetes than non-pregnant women (Goubau & Munyangeyo 1983) and these large numbers of spirochaetes may produce fever (Butler *et al.* 1979). Patients with a high spirochaetaemia are at greater risk of developing complications (Melkert 1987; Butler *et al.* 1979; Perine & Teklu 1983). In fatal cases miliary micro-abscesses have been found in the

Table 1. Age distribution and complications in 27 pregnant patients with relapsing fever

Maternal age (years)	No. of patients	Complications		
		None	Mild	Severe
15–19	8	4	2	2
20–24	8	5	1	2
25–29	5	2	2	1
30–34	3	2	1	—
35–39	2	1	1	—
40–44	1	—	—	1

Table 2. Gravidity and complications in 27 pregnant patients with relapsing fever

Gravidity	No. of patients	Complications		
		None	Mild	Severe
1	6	3	1	2
2–5	14	8	3	3
6–10	7	3	3	1

Table 3. Gestational age and complications in 27 patients with relapsing fever

Gestation at admission (months)	No. of patients	Complications		
		None	Mild	Severe
<7	7	3	2	2
7	7	6	1	—
>7	13	5	4	4

Table 4. Density of the spirochaetaemia and complications in 27 pregnant patients

Borrelia index	No. of patients	Complications		
		None	Mild	Severe
0·01–0·4	11	10	—	1
0·5–1·9	10	4	5	1
2 –10	6	—	2	4

spleen, frequently associated with necrosis in the mid-zonal regions of the liver. The heart may exhibit a myocarditis (Gillum 1976). Tick-borne relapsing fever can cause miscarriage or preterm birth.

The results of the present study are similar to those reported previously from Rwanda by Goubau & Munyangeyo (1983). We found a risk of 30% for pregnancy loss and a perinatal mortality of 15%, and the corresponding values in the Rwanda study were 33 and 15%, respectively.

Our results show a positive correlation between the degree of spirochaetaemia and the severity of complications, and the borrelia index was shown to have a prognostic value.

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Fetal supraventricular tachycardia: detection by routine auscultation and successful *in-utero* management. Case report

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Case report

A 26-year-old woman in her second pregnancy booked at 16 weeks gestation by dates which was confirmed both by serum human placental lactogen (hPL) (Whittaker *et al.* 1983) and a subsequent ultrasound scan at 17 weeks gestation (Campbell & Newman 1971). She was blood group O positive with no atypical antibodies; the VDRL was negative, she was immune to rubella and the haemoglobin concentration at booking was 12·4 g/dl. Her general health was excellent and there was no family history of heart disease.

The first pregnancy in 1984 had been complicated by placental abruption at 36 weeks gestation requiring delivery by emergency lower segment caesarean section. The infant, a male weighing 2·68 kg, was healthy and subsequent growth and development have been normal.

A vaginal delivery was anticipated on this occasion and she requested shared antenatal care with her general practitioner until review at the hospital at 28 weeks.

At review she was well and reported the pregnancy as uncomplicated. The fundal height measured 29 cm and the fetus was active with palpable vigorous movements. On routine auscultation with a Pinard stethoscope the fetal heart rate was noted to be very rapid with an estimated rate greater than 200 beats/min; this was confirmed using a Sonicaid FM6 electronic monitor.

The patient was admitted for investigation and more prolonged recording of the fetal heart. Cardiotocography showed episodes of sustained tachycardia in excess of 220 beats/min lasting for 5–10 min from a baseline rate of 120. The baseline variability remained between 5 and 10 beats/min but no accelerations were noted within the trace. There did not appear to be any association between the onset of the tachycardia and excess fetal activity.

Examination of the maternal cardiovascular system was normal with a regular heart rate of 80 beats/min, normal heart sounds and a blood pressure of 120/80 mmHg.

Fetal echocardiography was performed that evening by the paediatric cardiologists. This showed the presence of fetal supraventricular tachycardia (SVT) averaging 230 beats/min which was present for 90% of the recording time with the fetal heart rate returning to sinus rhythm for only a few minutes. (SVT was distinguished from other tachyarrhythmias such as atrial flutter or fibrillation by the confirmation of 1:1 atrio-ventricular activity on M-mode echocardiography.) The cardiac structure appeared to be entirely normal but there was some fetal ascites and a moderate pericardial effusion. These findings were suggestive of fetal cardiac failure and potential jeopardy but as the pregnancy had only reached 28 weeks it was considered prudent to maintain the fetus *in utero* for some time longer and offer medical treatment.

The fetus was treated by maternal digitalization commencing with 500 µg digoxin orally on the night of admission, twice daily the following day and daily thereafter. The patient remained in hospital for 24 h but as she had no adverse effects she was allowed home thereafter; a CTG before discharge revealed the same fetal heart pattern. The serum digoxin level was measured on the third day of treatment and a trough level of 1·5 µg/l was achieved.

A number of other investigations were undertaken to elucidate the possible aetiology of the SVT. Maternal blood was screened for the pres-

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ence of antinuclear antibody and lupus anticoagulant but neither was detected. A further detailed fetal ultrasound scan failed to show any structural abnormality but confirmed the presence of early hydrops. Skeletal growth was within the expected limits for gestation.

An amniocentesis was performed without difficulty and the amniotic fluid was sent for viral culture looking in particular for evidence of recent coxsackie or cytomegalovirus infection. These tests and cultures from the maternal nose, throat and faeces all failed to yield evidence of a possible pathogen. Repeat fetal echocardiography 3 days later suggested that SVT was present for 60% of the time and evidence of ascites and pericardial effusion persisted.

Despite 11 days of treatment with digoxin at confirmed therapeutic levels in maternal serum and a level of 0·2 μg in amniotic fluid (after 6 days treatment) the fetus still had a persistent SVT. Delivery was feasible at 30 weeks gestation but as there had been some response to medication it was decided to pursue the medical management policy for a little longer. It was decided to try an alternative anti-arrhythmic drug, and 110 mg (1·4 mg/kg) of flecainide were given intravenously over 5 min under maternal ECG and fetal echocardiographic control. There were no maternal side-effects and the fetus reverted to sinus rhythm almost immediately. She was maintained on oral flecainide, 100 mg three times daily, digoxin was withdrawn, and subsequent ultrasound scans showed that the fetus remained in sinus rhythm and the pericardial effusion and ascites progressively resolved.

Management was maintained on an outpatient basis thereafter and the pregnancy progressed uneventfully to 38 weeks when labour was induced by amniotomy and intravenous oxytocin. After a labour of 5 h and 10 min, during which the fetal heart remained normal on continuous monitoring by fetal scalp electrode, she had a vaginal delivery of a healthy female infant weighing 3455 g. The baby's heart rate remained regular with a rate of 140, there were no respiratory difficulties and no evidence of hydrops. An ECG performed twice the following day confirmed the continuation of sinus rhythm; no further medication was prescribed. They went home 48 h later and the baby has remained well.

Discussion

Fetal tachycardia may rarely represent a tachyarrhythmia causing cardiac failure and fetal death (Valerius & Jacobsen 1978). Such tachyarrhythmias have a reported frequency of about 1:20 000 pregnancies (Wladimiroff & Stewart 1985); this is probably an underestimate with many cases being undetected (Schreiner *et al.* 1978). The number of stillbirths and neonatal deaths attributable to non-immune hydrops (Anderson *et al.* 1983) appears to be increasing yet some of these cases may be responsive to medication (Lingman *et al.* 1980).

The aetiology of fetal tachyarrhythmias is varied, each with a different prognosis (Kleinman *et al.* 1982). A significant cardiac structural abnormality or tumour can be excluded by detailed ultrasonic screening. A definitive statement about the type of tachyarrhythmia can be made with M-mode echocardiography which will show the relative ratio between atrial and ventricular activity. In this way atrial flutter and fibrillation can be distinguished from supraventricular tachycardia as they have a variable conduction block while SVT is 1:1.

In the assessment of unexplained fetal hydrops, Nicolaides *et al.* (1985) found that a significant proportion of the fetuses had a variety of chromosomal abnormalities. It is thus important to ensure that the fetus has a normal karyotype before undertaking any invasive techniques designed to improve fetal outcome and particularly preterm caesarean section. It might even be suggested that a normal karyotype should be present before considering treatment of any kind although time might not allow this. Karyotyping is most usually undertaken on amniotic fluid cells and the culture of such cells can take up to 10 days before being suitable for cytogenetic analysis. Direct fetal blood sampling provides an answer in 2–3 days (Nicolaides *et al.* 1986) which is important if the fetus has already reached viability at the time of diagnosis or the hydropic change is already severe. This is not a technique that we have available routinely and should not be performed by the 'occasional' operator hence karyotyping was not done in our patient. We were reassured, however, by the normal cardiac structure and resolution of the hydrops with therapy.

Amniotic fluid was sent for viral studies as in-utero infection with cytomegalovirus and toxoplasmosis may cause cardiac conduction abnormalities although the mechanism is not clear. In this case no virus was isolated and the maternal serum titres were not indicative of recent infection. According to Lange *et al.*

(1982) the investigation of amniotic fluid may not be sufficient to exclude cytomegalovirus; a fetal blood sample assayed for a raised level of fetal IgM is better. Other abnormalities may be associated with intrauterine infection and these should be excluded if possible.

There is no agreement about the best method of treating fetal tachyarrhythmia but digoxin remains the drug of first choice (Wladimiroff & Stewart 1985; Bergmans *et al.* 1985). When this fails to control the heart rate, various agents such as beta-blockers, procainamide, verapamil, quinidine or amiodarone have been used either as additional or second-line therapy but the data are usually drawn from individual case reports (Wladimiroff & Stewart 1985). Amiodarone should be used with extreme caution as it contains a significant amount of iodine and may cause a fetal goitre (Burrow 1978). Knowledge about adverse effects of other cardiac agents is necessarily limited because of the rarity of their use; extrapolation is usually from knowledge of the drug effect in newborn infants. Unfortunately, little is known about specific drug actions in the fetus, and in particular the hydropic fetus, though it is reported that some agents like digoxin may have a particular affinity for the fetal myocardium (Wettrell 1976). Flecainide appears not to have been used during pregnancy before. It is a group-1 anti-arhythmic agent which is completely absorbed when taken orally and suitable for twice or thrice daily administration because of its slow metabolic clearance. It has been shown to be safe and effective in a variety of paediatric arrhythmias (Wren & Campbell 1987). In this case flecainide restored the fetal heart to sinus rhythm without any identifiable maternal or fetal side-effects.

Almost all fetal tachyarrhythmias reported in the literature have been identified during ultrasound examination to determine the cause of polyhydramnios (Gough *et al.* 1986) or non-immune hydrops (Nicolaides *et al.* 1985; Allan *et al.* 1986). This case and that of Lingman *et al.* (1980) demonstrate that routine auscultation during the antenatal period can reveal a tachyarrhythmia prior to the development of severe hydropic change. Prompt investigation and appropriate treatment can improve the outcome for these infants as at least 75% are likely to develop intrauterine or neonatal congestive cardiac failure or hypoxia (Bergmans *et al.* 1985). Those fetuses in whom non-immune hydrops is attributable to an arrhythmia would appear to have a better prognosis (Allan *et al.* 1986).

Some cases of unexplained intrauterine death may be caused by an unrecognized fetal arrhythmia. This case clearly demonstrates that the presence of fetal activity is not sufficient evidence of fetal well-being.

We would suggest that routine auscultation remains an essential part of antenatal care both in hospital and in the community.

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Protein C deficiency presenting as deep venous thrombosis in pregnancy. Case report

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Case report

A 21-year-old presented at hospital at 32 weeks gestation in her first pregnancy with a 2-day history of swelling, discomfort and inflammation of her left leg from ankle to groin. Apart from mild pregnancy-induced hypertension (blood pressure 150/96 mmHg) her pregnancy had been uncomplicated. She was a non-smoker and had never used oral contraception. She had no significant past medical history and no family history of thrombosis.

Clinical examination confirmed the presence of a left ileofemoral venous thrombosis. Venography was considered unnecessary. After blood samples for full blood count and for coagulation and thrombotic screening tests had been collected continuous intravenous heparin was started and adjusted to maintain an activated partial thromboplastin time (APTT) ratio of between 1·5 and 2·5.

The full blood count and platelet count taken before treatment were normal as were the coagulation screening tests. Pretreatment levels of plasma fibrinogen and antithrombin III were also normal. Protein C was assayed immunologically using an enzyme-linked immunosorbent assay (BCL Ltd, Lewes, UK) and functionally using an automated, amidolytic assay (McCall *et al.* 1987). Both assays revealed low protein C levels before heparin therapy; the protein C antigen level was 68% (normal range 73–120%) and the amidolytic activity 62% (normal range 74–128%).

After 10 days the intravenous infusion was discontinued and anticoagulation was maintained with self-administered subcutaneous heparin 8-hourly to maintain a trough APTT ratio of 1·5. The patient was discharged home at 35 weeks.

Labour was induced at term and a healthy boy weighing 3·76 kg was born after an uncomplicated labour. On the day of delivery 5000 i.u. heparin were given subcutaneously 12-hourly. Six hours after the delivery a continuous intravenous heparin infusion was started and the dose adjusted as before. On the third postnatal day warfarin was introduced. Full warfarinization was attained gradually over a period of several days. The heparin infusion was continued until the INR (international normalized ratio) was within the therapeutic range.

Reduced protein C levels were confirmed on four further blood samples before warfarin therapy was started. There were no statistically significant differences in the protein C levels between values at diagnosis (week 32) and those found during the next 5 weeks (Table 1).

Protein C assays were performed on blood samples collected from the patient's family. Protein C deficiency was found in her father and 18-year-old sister. Her mother and brother appeared normal (Fig. 1).

The amidolytic protein C activity of a cord blood sample from her son was 15%, slightly lower than the normal cord blood range of 18–44%. As the baby was a healthy full-term infant no specific treatment other than a routine single dose of intramuscular vitamin K was given. At 15 weeks of age the baby's protein C on amidolytic assay of a venous blood sample was 38%. Follow-up of the baby has been arranged.

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Table 1. Protein C levels before introduction of warfarin therapy

	Gestation (weeks)				
	32	34	35	36	37
Protein C level (%)					
Antigen	68		62	58	62
Amidolytic	62	59	55	58	55

Discussion

Thromboembolism is the commonest cause of mortality associated with pregnancy. Whilst physiological alterations in the coagulation and fibrinolytic systems occurring during pregnancy result in increased coagulability (Dewhurst 1981) it is postulated that thrombosis only occurs if there is some added 'risk' factor.

Increasingly it is becoming recognized that this added 'risk' factor may be a hereditary or acquired disorder of haemostasis. Most clinicians are already aware of the risks of antithrombin III (ATIII) deficiency and there are many reports of thrombosis occurring in pregnancy in women with ATIII deficiency (Hellgren *et al.* 1982; Samson *et al.* 1984). Less has been written about the risks of pregnancy in protein C deficiency.

Protein C is a vitamin K-dependent protease (Stenflo 1976) which is activated by thrombin (Kisiel 1979). Activated protein C acts as an anticoagulant by inactivating factors Va and VIIIa (Marlar *et al.* 1982) and also has profibrinolytic activity (von Hinsbergh *et al.* 1985). The absence of protein C means there is continued activation of coagulation and a reduction in fibrinolysis with an increased likelihood of thrombosis.

Protein C deficiency may be congenital or acquired when it is associated with liver disease (Mannucci & Vigano 1982), disseminated intravascular coagulation (Griffin *et al.* 1982) and postoperative states. Protein C is also reduced postpartum. Conversely, it is normally increased during pregnancy (Mannucci *et al.* 1984). In healthy full-term infants protein C levels are about one-third of normal adult values. This probably reflects partial liver immaturity in the newborn (Polack *et al.* 1984). By 6 months of age infants normally have 'adult' protein C levels (Andrew *et al.* 1985).

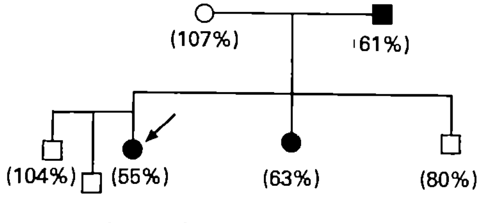

Fig. 1. Pedigree of patient's family. The figures in parenthesis show protein C values by functional assay. ○ □, Normal; ● ■, low protein C; ←, propositus.

Congenital protein C deficiency, inherited as an autosomal dominant with incomplete penetrance, has been reported in both heterozygotes and homozygotes. Reported cases of homozygous protein C deficiency in the newborn have been characterized by extremely low protein C levels (<6%) and a syndrome of chronic relapsing purpura fulminans (Estelles *et al.* 1984; Branson *et al.* 1983).

The clinical severity of heterozygous protein C deficiency seems to be variable. Families reported in early papers appear to have suffered spontaneous and sometimes recurrent venous thromboembolism (Broekmans *et al.* 1983*a*; Griffin *et al.* 1981). Other families have been reported in which thrombosis seems to have occurred only in association with some precipitating event such as pregnancy or surgery (Horrellou *et al.* 1984). As the assay for protein C has become more widely available, increasing numbers of asymptomatic family members have also been reported (Estelles *et al.* 1984; Branson *et al.* 1983; Gonzalez *et al.* 1985).

Management of patients with protein C deficiency requires careful and individual consideration. Obviously where thrombosis is the clinical problem this must be treated, usually initially with continuous intravenous heparin. In patients who are not pregnant, oral anticoagulation should be introduced after 5–10 days heparin. However, care must be taken with the introduction of vitamin K antagonists since severe skin necrosis has been reported during this phase in protein C-deficient subjects (Broekmans *et al.* 1983*b*). Oral anticoagulation must be introduced gradually under full heparin cover. In pregnant women self-administered subcutaneous heparin may be preferred to oral anticoagulants and can be started after an initial period of intravenous therapy.

Patients who have a history of recurrent venous thromboembolism probably require long-term anticoagulation. However, it is becoming clear that patients who are protein C deficient may be at risk of developing severe skin necrosis not only at the time of induction of therapy but also at times when their anticoagulant control is poor (Teepe *et al.* 1986). Careful monitoring of anticoagulant therapy in such patients is therefore mandatory. The plasma levels of protein C increase in response to anabolic steroids such as stanozolol and danazol (Preston *et al.* 1983; Gonzalez *et al.* 1985). Although these agents may have potential therapeutic value in some protein C-deficient patients, in practical terms they are of very limited usefulness in congenital protein C deficiency.

Patients known to be protein C deficient should be offered prophylactic anticoagulation to cover situations of increased thrombotic risk, in particular surgery and pregnancy. Anticoagulation should probably continue for 3–6 months after removal of the risk.

It is extremely important that a patient found to be protein C deficient understands not only the immediate management but is also fully appraised of possible future problems and advised how best to avoid them. It is also most important that all family members are encouraged to present themselves for protein C assays. Asymptomatic protein C-deficient subjects should be counselled about the advisability of prophylactic anticoagulation to cover thrombotic risk situations.

As the finding of a haemostatic abnormality has serious implications, both for the individual concerned and for other family members, it is essential that women who develop thrombosis in pregnancy, particularly in early pregnancy or in the puerperium, are fully investigated.

Acknowledgment

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SHORT COMMUNICATIONS

Uteroplacental perfusion after epidural analgesia for elective caesarean section

M. G. Long *Senior House Officer,* M. Price* *Registrar* & J. A. D. Spencer *Senior Lecturer and Consultant, Royal Postgraduate Medical School Institute of Obstetrics and Gynaecology, University of London, and *Department of Anaesthetics, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG, UK*

A recent Doppler ultrasound study concluded that epidural analgesia improved uteroplacental perfusion (Giles *et al.* 1987) whereas a previous radio-isotope study had suggested that intervillous blood flow may decrease slightly, though not significantly, with epidural analgesia (Jouppila *et al.* 1978). We report an assessment of the variability of measurements of the uteroplacental blood flow pulsatility index (PI) and the results of a study of the effects of fluid preloading and epidural analgesia on uteroplacental perfusion in women undergoing elective caesarean section.

Subjects and methods

The variability of measurements of the uteroplacental PI in late pregnancy was assessed in a group of 20 women resting in a semi-recumbent position. Each had three recordings made from uteroplacental vessels on the placental side of the uterus using a 4 MHz, low power, continuous-wave system Doppler ultrasound system (Vasoflo-3, Oxford Sonicaid Ltd, Chichester, UK). Each value of PI (maximum systolic minus minimum diastolic frequency divided by the mean frequency of the maximum frequency envelope, Gosling & King 1975) was derived from six consecutive waveforms on each record and the standard deviation (SD) of values from the three records on each woman was calculated for the analysis of variance. The coefficient of variation was obtained by expressing the square root of the mean of the 20 SD^2 values as a percentage of the overall mean of the 60 values of the PI.

Twenty women with uncomplicated singleton pregnancies undergoing elective caesarean section at term had Doppler ultrasound measurements of uteroplacental perfusion taken before and after intravenous fluid preloading (1·0 litre Hartmann's solution) and 15 and 30 min after insertion of 0·5% bupivicaine into the epidural space. Intermittent readings of systolic and diastolic blood pressure were taken from the arm. Mean values of PI, blood pressure and heart rate after preloading and after the epidural were compared with the initial resting value using Student's paired *t*-test. The readings after the epidural were also compared with those after the fluid preload.

Results

The mean PI of the uteroplacental circulation in the variability study was 0·63 (SD 0·17) and the coefficient of variation for the 60 measurements was 6%.

The mean uteroplacental PI at rest in the epidural group was 0·67 (SD 0·22) and Table 1 shows that there were no significant changes in mean PI, blood pressure or heart rate following intravenous fluid preloading or at 15 and 30 min after the epidural. Two women were given ephedrine because of hypotension immediately following their epidural.

Discussion

A coefficient of variability of 6% for measurements of the uteroplacental PI compares well with previous reports which have been as high as 17% (Rightmire & Campbell 1987).

Our findings after epidural differ from those of Giles *et al.* (1987) who reported significant

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Correspondence: J. A. D. Spencer

Table 1. Effects of fluid preload and epidural anaesthesia on the maternal cardiovascular system of 20 women undergoing elective caesarean section

	Fluid preload		Time after epidural	
	Before	After	15 min	30 min
Uteroplacental pulsatility index	0·67 (0·22)	0·68 (0·18)	0·68 (0·12)	0·67 (0·13)
Blood pressure (mmHg)				
Systolic	117 (17·7)	109 (14·9)	109 (9.2)	119 (14·7)
Diastolic	76 (15·6)	76 (12·3)	66 (9·5)	77 (9·5)
Maternal heart rate (beats/min)	95 (13·8)	91 (11·8)	92 (12·4)	93 (15·5)

Values are mean (SD).

falls in both blood pressure and the ratio of maximum systolic to end diastolic frequencies (A/B ratio). They concluded that resistance to placental perfusion decreased following the epidural but this assumes that the reduction in the A/B ratio resulted from an increase in frequencies at the end of diastole. It is possible that the fall in the A/B ratio resulted from a reduction in maximum systolic frequencies due to the fall in blood pressure and may not have indicated an improvement in uteroplacental perfusion. In fact the fall in the A/B ratio began before the epidural after expansion of the circulating blood volume and this is difficult to explain in the absence of significant changes in pulse and blood pressure unless changes in viscosity affect systolic and diastolic velocities differently. We found no change in PI after an identical fluid preload.

The uteroplacental circulation is believed to be maximally dilated under normal circumstances, and falls in blood pressure cause a reduction in uterine perfusion (Greiss 1967). Two women in our study had significant hypotension after the epidural but neither showed changes in the PI after treatment with ephedrine. Our findings therefore agree with those of Jouppila *et al.* (1978) and experimental data derived from pregnant ewes (Wallis *et al.* 1976), in suggesting that, in the absence of changes in blood pressure, epidural analgesia has no significant effect on uteroplacental perfusion.

Acknowledgments

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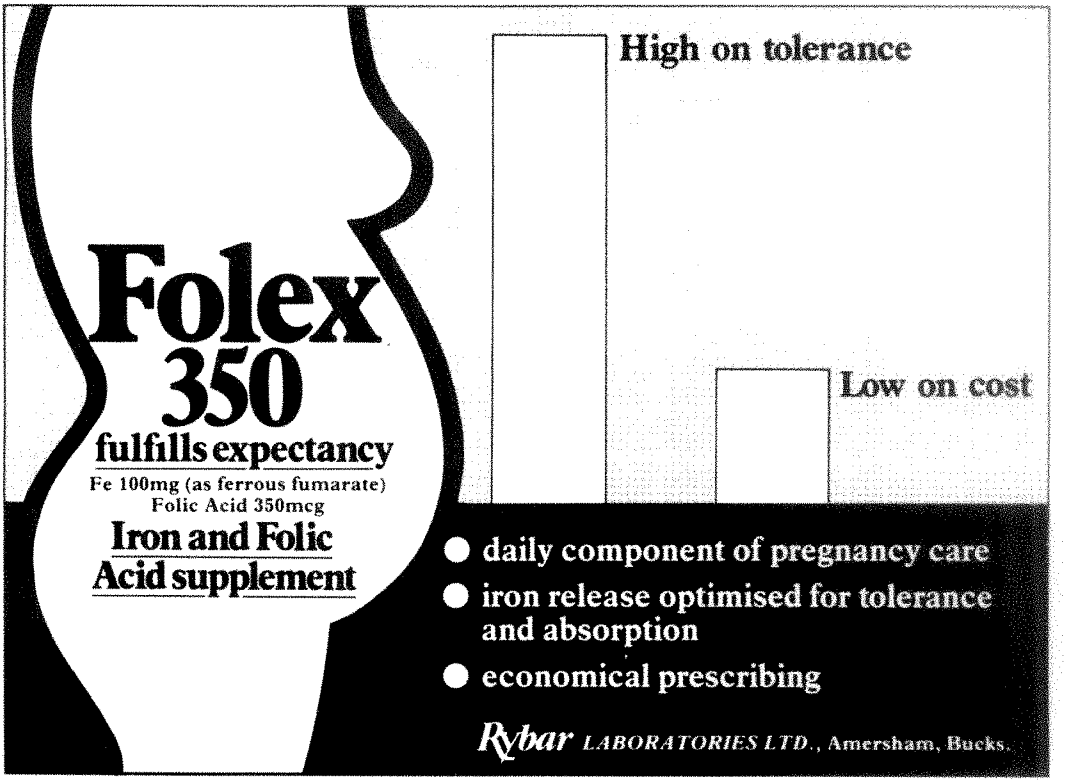

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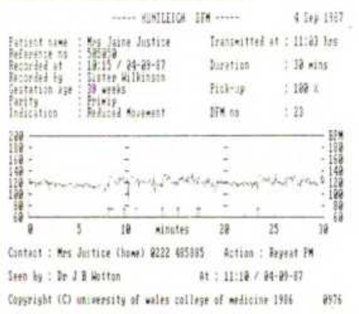

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Recorded by    : Sister Wilkinson
Gestation age  : 30 weeks                Pick-up        : 100 %
Parity         : Primip
Indication     : Reduced Movement        DFM no         : 23

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British Journal of Obstetrics and Gynaecology
October 1988, Vol. 95, pp. 1083–1084

CORRESPONDENCE

Candidiasis in women fitted with an intrauterine contraceptive device

Dear Sir,

Dr Parewijck and colleagues [*Br J Obstet Gynaecol* (1988) **95,** 408–410] found a statistically significant difference in the prevalence of vaginal colonization by *Candida* spp. between women using an intrauterine contraceptive device (IUCD) and non-users. A statistically significant difference between the two groups was also seen for symptomatic candidiasis.

These findings are at variance with those of other papers quoted by Dr Parewijck and colleagues in the discussion, so that their conclusion seems to be an original contribution. The weakness of their report is that the features of the two groups (IUCD-users and control group) are not analysed, therefore it is not possible to say whether the two groups are comparable. One important difference between the two groups is nonetheless evident: the women in the control group were neither wearing an IUCD nor on steroid contraception, it is therefore reasonable to assume that some used barrier contraception (which is probably associated with less frequent sexual intercourse than other methods) and others had no partner at the time of the investigation.

The contribution of sexual transmission to candidiasis has been appreciated as far as balanitis is concerned (Oriel *et al.* 1972; Drake *et al.* 1980). Endogenous flora are probably responsible for most cases of vaginitis. Whether the male partners of women with *Candida* vaginitis should be treated in order to reduce the frequency of recurrence is controversial. Bisschop *et al.* (1986) could not show any benefit in a double-blind randomized study in which male partners were simultaneously treated. The Nystatin Multicenter Study Group (1986) found that clearance of the intestinal *Candida* reservoir in women with vulvovaginitis was associated with a higher cure rate.

It is possible that sexual intercourse facilitates vaginal candidiasis by transfer of flora from the female external genitalia into the vagina and by microtrauma. A comparison between groups of women of the same age and sexual behaviour should be made before any conclusion on the causative role of IUCDs could be drawn: it might not be possible at all because groups using different contraceptive methods tend to differ in many respects.

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Authors' reply

Dear Sir,

We thank Dr Bignardi for his comments. The two groups compared were really very similar: all the women investigated were sexually active and the mean age in the IUCD group (36·1 years) was similar to that in the control group (38·7 years) which suggests comparable levels of sexual activity. The assumption that an important proportion of the women in the control group was relying on barrier contraception (not popular in Belgium) or had no partner at the time of investigation must be contradicted. As already mentioned, all control subjects were sexually active and only 8% used condoms. The others had been sterilized (51%) or had had a hysterectomy (6%), used rhythm methods or/and coitus interruptus (32%) or no method at all (3%).

An important finding of the study is that positive *Candida* cultures were significantly more frequent on the IUCD than in the vaginal contents. This strengthens the concept that the foreign body (IUCD) acts as a reservoir for micro-organisms suggesting that the lower bacterial clearance capacity rate of IUCD users—demonstrated for *Actinomyces* sp.—may be extended to yeasts.

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BOOK REVIEWS

In Vitro Fertilisation: Past, Present and Future edited by S. Fishel & E. M. Symonds. IRL Press, Oxford, Washington DC, 275 pages with figures and tables, £17.50, US$ 50.00.

Inevitably books on IVF have a short shelf-life. The standard formula appears to be chapters on historical, methodological, clinical, legal, ethical, and social aspects, usually in that order. So in some ways this book is no different from several before. Nevertheless, it does fairly reflect the state of the art in 1986, when Simon Fishel and John Webster were setting up their private clinic in Nottingham. Many of the chapters are written by recognized experts, Jones on clinical aspects, Hodgen (with van Uem) on follicular growth and ovulation, Wickland on oocyte recovery, Aitken on semen, Dunstan on ethics; and although the titles may have a familiar ring, there is nevertheless much new information. Perhaps the weakest chapters are those on endocrine evaluation, and pregnancy outcome (with particular respect to multiple births). Leo Abse contributes a most interesting chapter on politics which is as relevant for its insights into the parliamentary process as much as Mr Abse himself.

As in previous books, there is much important detail on clinical and embryological aspects which will interest those setting up programmes and clinics. All in all this book represents good value. Most practitioners will probably have a copy already and it will do well until the next book comes along.

Allan Templeton

Birthright: What Every Woman Needs to Know: Facts and Fears about Pregnancy, Childbirth and Womanhood edited by Penny Junor. Century paperbacks, London, 1988, 203 pages, £5.95

With the fanfare of a foreword by the Princess of Wales, this summary of information from 24 distinguished authorities not only shows the benefits of recent research into important aspects of pregnancy, childbirth, and gynaecological disorder (some of it achieved by the RCOG's own charity Birthright) but also points to those areas where further support is likely to bear fruit. The science, study and remedy of infertility, miscarriage, and major pregnancy disorders form the greater part with easy reading which is completely up to date. Gynaecological themes also presented include menstrual disorders, menopause, incontinence, pain, cervical screening and its significance, and the surgery of women's pelvic cancers.

The authors set out their current knowledge in the form they would choose for an ideal consultation. Though some have not altogether escaped the habit of writing a textbook chapter (though couched in easy terms), the majority have responded to the editor's urging to use a question/answer format; their confidence that they know what a woman wants to know distinguishes those clinicians with vast experience of counselling in their fast-changing specialty.

Is the writing such that every woman will understand? For a past generation of consultants it was sources such as *Woman's Hour* on the car radio between clinics that told them what they never learned about women's needs in their training—about postnatal guilt for lack of stereotype maternal feelings, for example. (Incidentally, postnatal depression isn't included in this first edition!) Today's doctors have learned and listen better; science is more shareable than mystique, and the language barriers are nearly down.

This is a book for every woman and every couple to share, for there is no aspect in it of woman's health that does not touch on the understanding of her partner. A bedside book, even, for another side to caring?

Anthony Alment

Drugs and Pregnancy: Human Teratogenesis and Related Problems Second edition edited by D. F. Hawkins. Churchill Livingstone, Edinburgh, 1987, 349 pages with figures and tables, £17.50

What substantial news does this second edition provide?

In section one, the exceptionally well-written chapter by F. Reynolds covering the placental transfer of drugs has been enlarged and now contains some additional important comments on the relative affinities of maternal and fetal plasma for drugs, the role of protein binding on fetal–maternal drug concentration ratios, and the possible consequences on rate and duration of neonatal effects. The discussion on the general principles of drug prescribing in pregnancy presents with a fresh estimate: probably fewer than 1% (and not 3!) of developmental defects are caused by drugs, but harm to the fetus may result from omission of treatment and may give rise to liability.

In section two, the *plat de résistance* is undoubtedly the chapter 'Drug Treatment of Medical Disorders during Pregnancy' which has been updated. Anticoagulants are described in more detail, in particular their use in women who have had heart valve replacement, and there is a table added on fetal and neonatal complications associated with these drugs. There are some short remarks on cardiac drugs—unfortunately ending with a rather odd, incomplete sentence, due to typographical error. For dermatological conditions information presented in five lines on retinoids seems totally insufficient. There are a few new 'problem-oriented' chapters, including one on antiemetics which clearly makes the point that so far there is no valid evidence that these drugs are teratogenic. Another contribution on 'Drugs in Labour' should rather be headed 'Management of Pain and Distress'. Anticonvulsants, formerly included in P. F. Hawkins's chapter, are now discussed in more detail by P. G. Palmer, together with sedatives. Special emphasis is given to some classics such as barbiturate, phenytoin, carbamazepine, and valproic acid.

Little news either for antimicrobials except that a 5-day course (instead of 10 days) of a short-acting sulfonamide or amoxycillin is advocated for the treatment of bacteriuria with more than 10^5 micro-organisms/ml. With premature rupture of the membranes the risk of fetal and neonatal infection is not reduced by the prophylactic use of antibiotics. In the chapter on preterm labour it is stressed, at last, that oral administration of β-sympathicomimetics does not convincingly stop uterine contractions. There is an interesting adjunct to the chapter on corticosteroids concerning the 'lupus anticoagulant' which is probably the same as, or closely resembles, anticardiolipin antibody. It is reported that in patients with a history of recurrent early or late fetal loss whose serum contains this antibody, successful pregnancy can be achieved by suppressing the antibody with large doses of prednisone (and small doses of aspirin) throughout pregnancy. (Maybe!) The last chapter, 'Breast Feeding and Drug Treatment', offers few changes, but includes, in my view, a rather misleading remark that when the baby of a nursing epileptic mother who is on anticonvulsants gets sleepy it is possible to test whether or not these drugs are responsible by giving artificial feeding just for 24 hours. Indeed, a much longer period may be necessary when drugs are involved whose half-lives are 60–100 (or more) hours.

Comparing this second edition with the first it is all too obvious that while new drugs constantly appear on the market hard facts about them follow only slowly. Positive guidance on how to be cautious when using them, especially in the delicate and complicated situation of pregnancy, thus becomes an almost unreachable goal. Nevertheless, in this slim volume a number of specialists have joined their efforts to present up-to-date information which may be useful to all who wish a rapid overview when they face the difficult task of treating pregnant patients.

Béatrice Krauer

Oxford Reviews of Reproductive Biology Volume 9 edited by J. R. Clarke. Oxford University Press, Oxford, 1987, 504 pages with figures and tables, £50.00

'Oxford' in the title gives stature to an academic book, just as the prefix 'AA' lends a cachet to a gazetteer. The word 'Oxford' is a trade mark (a fact that must cause headaches to the people who publish the local bus timetables) and it is easy to see why it is carefully guarded, as it describes a long tradition of scholarship, uncompromising without being inaccessible. This series of *Reviews of Reproductive Biology* has lived up to its adjective and the latest volume is as good as its predecessors. The editor—with the help, no doubt, of his distinguished non-Oxonian editorial board—has shown remarkable skill in soliciting contributions from authorities who have worked in their fields long enough to produce mature overviews but not so long as to grow stale. For example, a chapter on inhibin is contributed by Dr de Jong of Rotterdam, and Iain

Clarke writes from Melbourne on GnRH and ovarian hormone feedback. The main emphasis of the book is on scientific aspects of endocrinology: obstetricians may be tempted to skip the chapter on ovulation in the hen but will be better pleased by the clinical orientation of Professor Rodeck's review of chorionic villus biopsy. The thin dividing line between today's research and tomorrow's practice, however, is well illustrated by the inclusion of a chapter on neural transplants—a technique now familiar to viewers of the *Six o'Clock News* but as far as reproduction is concerned still restricted to mice. The book's wide range of topics—from prostatic cancer to opioid peptides and the structural changes of fertilization—is ideal for the library, though it means a lot of excess weight for the individual borrower interested in only one subject. But will today's computerized library technology ensure that all potential consumers are made aware of this varied feast of information while it is still warm?

J. Drife

The Status of the Human Embryo edited by G. R. Dunstan & M. J. Sellars. Oxford University Press/King Edward's Hospital Fund for London, 1988, 119 pages with figures and tables, £15.50

For those involved in human embryo research, it has been painfully obvious for some time that there is an uphill struggle to educate the public and Parliament. There has been a good deal of misinformation, which because of its sensationalist nature, has been emphasized by the press. This book, written by a small group who have been meeting over the years at King's College, London, to discuss topics on medical ethics, attempts to assess the moral status of the human embryo and the liberties we are entitled to take.

The book is a timely publication in the light of probable legislation following firstly the Warnock Report and then the White Paper at the end of 1987. It sets out the questions, gives some basic facts about embryology, details the medical and scientific reasons for embryo research, and then examines the status of the human embryo in various circumstances and from the standpoint of several moral traditions. The book does not attempt to draw conclusions, but rather to reflect the views of individuals, and through them organizations, who have an interest in this concern. Following introductory chapters which state the problems and examine the biology of the embryo, the reasons for wishing to perform embryo research are given. Although clearly written for a lay audience, some of the reasons were, I felt, a little vague, and the chapter as a whole did not emphasize the importance of the investigation of the basic process of conception in our own species, which is, after all, a major part of what has come to be called embryo research. In a learned chapter, G. R. Dunstan recalls Western moral tradition and points out that absolute protection of the human embryo is indeed a novelty although there were many penalties of varying severity for destruction of the developing fetus. The old Irish canons, for example, had three stages: 'after it is established in the womb (3½ years)'; 'if the flesh is formed (7 years)'; and 'if the soul has entered it (14 years)'; the time in brackets being the sentence on bread and water for offenders. In the next chapter John Marshall argues effectively that the development of the nervous system, and hence the ability of the embryo to sense pain, cannot be taken as a suitable point for limiting experimentation. The Jewish tradition is carefully examined by Sir Immanuel Jacobovits who implies that the full state of personhood is not acquired until after birth. He feels that there is some justification for the use of spare embryos for research, but feels these considerations are limited compared to the importance of the status of the family and the essential interests of the child. In this respect he takes a rather hard line on the practice of AID, pointing out that children facing uncertainties in seeking their origins are at grave disadvantage in building social and morally stable lives. The Roman Catholic viewpoint is well known, but what is of interest is Brendan Sloane's admission that the present view could be based not so much on a certainty that ensoulment occurs at fertilization, but rather that, as we cannot know, it is best to err on the side of safety. The final chapter is a scholarly dissertation by Peter Byrne on the animation tradition in contemporary philosophy. This is perhaps the most difficult chapter to follow, but is worth pursuing. It underlines the importance of personhood, but emphasizes the difficulties in interpretation of a social as opposed to a biological concept of personhood. In many ways this is the nub of the issue and merits further consideration.

I hope that many will read this book, whatever their current views. It is particularly important that Parliament considers these issues carefully before legislation is introduced.

Allan Templeton

Books received some of which may be reviewed at a later date

The Menopause edited by John W. W. Studd & Malcolm I. Whitehead. Blackwell Scientific publications, Oxford, 1988, 312 pages with figures and tables, £49.50

Illustrated Textbook of Obstetrics by Geoffrey Chamberlain, Charlotte Gibbings & John Dewhurst. Gower Medical Publishing, London, 1988, 263 pages, £13.50

Prenatal and Perinatal Biology and Medicine Vol. 1: Physiology and Growth edited by Norman Kretchmer, Edward J. Quilligan & John D. Johnson. Harwood Academic Publishers, New York, 1988, 350 pages, $70.00

Female Genital Cancer edited by S. B. Gusberg, Hugh M. Shingleton & Gunter Deppe. Churchill Livingstone, New York, Edinburgh, London, 1988, 857 pages with figures and tables, £95.00

Clinical Preventive Medicine: Health Promotion and Disease Prevention edited by T. Warner Hudson, Michael Reinhart, Steven D. Rose & Gary K. Stewart. Little, Brown & Company, Boston/Toronto, 1988, 696 pages with figures and tables, £19.95

The Penguin Medical Encyclopaedia Third edition by Peter Wingate with Richard Wingate. Penguin Books Ltd, London, 1988, £5.95

Medical Counselling Before Pregnancy edited by Dorothy Reycroft Hollingsworth & Robert Resnik. Churchill Livingstone, New York, Edinburgh, London, 1988, 551 pages with figures and tables, £50.00

Management of Common Problems in Obstetrics and Gynecology Second edition edited by Daniel R. Mishell Jr & Paul F. Brenner. Medical Economic Books, Oradell, New Jersey, 1988, 699 pages with figures and tables, no price given

Computing for Clinicians by Tim Chard. Elmore-Chard, London, distributed by IOP Publishing Ltd, Bristol, 1988, 136 pages with figures and tables, £18.00/$32.00

Infertility Services: A Desperate Case by Naomi Pfeffer & Allison Quick. Available from The Greater London Association of Community Health Councils, 100 Park Village East, London NW1 3SR, £5.00 (incl. postage)

World Health Statistics Quarterly Report: Disease Prevention and Control Vol. 41 No. 2 1988. World Health Organization, Geneva, Sw. fr. 23

Proceedings and Symposia

Biology of Human Milk (Nestlé Nutrition Workshop Series, Vol. 15) edited by Lars A. Hanson. Raven Press, New York, 1988, 247 pages, $61.50

Vitamins and Minerals in Pregnancy and Lactation (Nestlé Nutrition Workshop Series, Vol. 16) Joint Symposium Nestlé–Hoffmann-La Roche, edited by Heribert Berger. Raven Press, New York, 1988, 478 pages, $61.50

Role of Prolactin in Human Reproduction First, second and third symposia on Physiological Human Reproduction and Prolactin, Tokyo, 1985/86/87, edited by M. Mizuno, H. Mori & Y. Taketani. Karger, Basel, 314 pages with figures and tables, £117.80

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Altman, D. G , Gore, S M., Gardner, M J & Pocock, S. J. (1983) Statistical guidelines for contributors to medical journals *Br Med J* **286**, 1489–1493

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COMMENTARY

Does HPV cause cervical cancer?

The relation between human papillomavirus (HPV) and cervical cancer is unquestionably one of the 'hot topics' in current gynaecological research. The issue is important and part of its importance is that it bears on a high profile aspect of our practice. As usual in a controversy of this nature we have a spectrum of opinions from the enthusiasts for HPV causation through the baffled observer to the cynics who regard the association as purely casual. The fact is, that at the moment we simply do not know whether or not HPV causes cervical cancer. What we can do, however, is to review the data and attempt to develop a reasoned assessment.

Epidemiological studies indicate that the principal risk factors which have so far been identified for cervical neoplasia are early onset sexual activity, multiple partners and cigarette smoking. The obvious interpretation of these data is that there requires to be the opportunity for one or more mutagens to be sexually transmitted and that long-term exposure to a carcinogen such as cigarette tar encourages promotion through precancer to invasive disease. This is over-simplistic as many women who develop cervical cancer are non-smoking and have had only one sexual partner; indeed the connotation of cervical cancer as an affliction of promiscuous women has been unfortunate and has resulted in some women feeling that they have 'caught' the disease.

Within a hypothesis of this nature HPV appears an excellent candidate as a causative agent in cervical neoplasia. It is sexually transmitted, commonly causes infection of the lower genital tract and its stigmata are frequently seen in association with pre-invasive neoplasia (Meisels *et al.* 1981). The advent of recombinant DNA technology in molecular biology, with its ability to identify viral DNA in host tissues spotlighted HPV as a possible progenitor of cervical cancer. Not only were HPV genomes discovered in cervical intraepithelial neoplasia (CIN) and cancers but different subtypes were discovered in different grades of the disease. HPV-6, originally isolated from the classical genital wart condyloma acuminatum, appeared to be associated with CIN I whereas a majority of CIN III and invasive disease appeared to contain HPV-16 genomes (Crum *et al.* 1984). This finding was confirmed by McCance *et al.* (1985) who also suggested that whereas HPV-6 was extrachromosomal, HPV-16 was integrated into the host cell genome. More recently, study of HPV gene expression has shown that HPV-16 encodes a protein mapped in the region of the E6/E7 open reading frames which will co-operate with an activated *ras* oncogene to transform primary cells (Matlashewski *et al.* 1987). Integration of a papillomavirus in the host chromosomes, by a subtype known to be associated with high-grade CIN and invasive cancer, and with expression of transforming viral genes, which in addition satisfied some of the epidemiological requirements for the disease, is regarded by some as sufficient evidence to incriminate HPV-16 as the cause. This concept of a sexually transmitted virus causing cervical cancer, which itself 'enjoys' a high political profile (dwarfed only by AIDS), has been seized upon by the popular press and has pre-empted more considered appraisal of the data.

Rather neglected until recently has been the question of HPV in control tissues in studies of this type. In trying to assess the relevance of HPV in cervical neoplastic tissues it is important to have knowledge of its presence both in normal areas of the affected cervix and the cervical tissues of women who have never had cervical disease and whose cervix is histologically normal.

Recently published data from Glasgow (Murdoch *et al.* 1988) have indicated that 60% of biopsies from normal areas of the transformation zone of women with CIN contained HPV 16 genomes; moreover, this DNA was frequently integrated and in higher copy number than in the pre-cancerous biopsies.

Meanwell *et al.* (1987) have studied some normal subjects in the West Midlands. They probed native or original squamous epithelium of the cervix of women with no history of genital warts and whose cervices were normal by colposcopy and cytology. HPV-16 DNA was identified in 5 of 16 such tissues, though in this report the viral DNA was not integrated in the host chromosome. These authors, who also reported on malignant tissues (65% of cervical tumours contained HPV-16 DNA), stated that stepwise discriminant analysis of the data demonstrated that the presence of HPV in cervical tissue is a function of increasing age, with the resultant opportunity for infection, and was not preferentially found in neoplastic tissue.

These data suggest that HPV-16 may be a harmless passenger in cervical cells of most women, but do not refute the possibility that in some individuals HPV-16 may initiate neoplastic change.

In trying to attribute causality to HPV in this confused situation, we must know more of the natural history of HPV infection, both at the level of histological change and HPV genomes in host cells. A study from Melbourne (Mitchell *et al.* 1986) showed that cytological evidence of HPV infection in otherwise normal smears conferred an increased relative risk of 15 for CIN developing over a 6-year period. Singer's group (Campion *et al.* 1986) attempted to assess the potential of HPV for inducing malignant change by probing lesions that appeared to progress from CIN I to CIN III over an 18-month period. They found that 85% of these lesions contained HPV-16 and suggested that the presence of HPV-16 indicates a high risk of rapid progression. Although interesting, these findings are of a circumstantial nature and it should be noted that the CIN I at the beginning of the study was assessed by colposcopy and cytology which themselves are unreliable without biopsy.

The HPV story has been an exciting episode in gynaecological cancer research but there is a danger of too much significance being ascribed to the presence of HPV in the cervix. Some have suggested, for example, that we should screen women for cervical infection with HPV-16. While its presence is a marker for women at increased risk of cervical neoplasia and evidence for its involvement in the disease is impressive, HPV cannot yet be designated a causal factor because the evidence is essentially circumstantial. Widespread use of a screening method for HPV-16 in smears would not be a useful development because it would be neither a specific nor sensitive test for pre-cancer and would not obviate the need for cytology. Many women would be involved in unnecessary surveillance (at least 10% of those screened according to de Villiers *et al.* (1987)), many of whom would feel tarnished by being told they have a sexually transmitted infection, and if colposcopy were to be advocated for HPV-16 positive women, existing services would be hopelessly overstretched.

Carcinogenesis is regarded as a multifactorial multistage process which well fits the epidemiology of cervical cancer. We have few firm data to indicate which other factors may be involved though studies have suggested that long-term use of oral contraception (Vessey *et al.* 1983) and heavy smoking (Harris *et al.* 1980) confer increased relative risk. Ten years ago herpes simplex virus (HSV) was felt almost certainly to be involved, until it became apparent that HSV DNA was not often detectable in neoplasms. Many have since discarded HSV as a credible aetiological agent, although its oncogenic potential and venereal transmission suggest that it should not be disregarded. Other environmental carcinogens and as yet unknown processes which are presumably crucial in causing other common epithelial cancers are probably also of great importance in cervical cancer. Though cervical carcinogenesis may ultimately proceed via common molecular pathways the relative importance of various agents inducing such change may be different for different tumours; HPV infection is perhaps the initiating factor in a proportion of cases.

Research has reached a rather frustrating position where available evidence suggests causality but conclusive proof is lacking. Future efforts at defining the role of HPV should be directed at determining what effect HPV exerts on cervical cells thus progressing beyond its mere association with neoplasia. There are three obvious avenues towards this objective. The first is to develop an in-vitro model which would permit study of HPV/cervical cell interactions. Some progress has recently been made in this area using a foreskin keratinocyte cell culture model in which it has been possible to achieve HPV infection and to observe features which suggest at least partial transformation (D. J. McCance *et* *al.* personal communication BSCCP 1988). The second approach would be to investigate animal models which could permit observation of the effects HPV can exert *in vivo*. A nude mouse system has been described (Kreider *et al.* 1985) where cervix tissue grafted under the renal capsule undergoes dysplastic change following exposure to a cell-free extract of condyloma acuminatum. Such experimental methods could be further exploited to study what effect HPV exerts on cellular genes and their expression. The third research strategy, and in some respects the most important, is long-term longitudinal study of large cohorts of women with evidence of HPV infection. Two potential groups of interest are those women in whom colposcopy and biopsy have demonstrated 'virus infection' and those with normal cytology in whom screening of cervical cells by DNA/DNA hybridization (perhaps with the aid of DNA amplification) has indicated HPV-16 genomes. This would not only provide a valuable contribution to the investigation of cervical carcinogenesis but will provide much needed information about the natural history of HPV infection of the cervix, as well as guidance about how best to manage these women.

In a similar commentary 3 years ago Singer & McCance (1985) suggested that the case for HPV causation could best be made if vaccination against HPV reduced the incidence of cervical cancer amongst those at greatest risk. It would not be appropriate to pursue such a vaccine programme until it could be shown conclusively that HPV was an essential factor. The present Commentary posed the question—does HPV cause cervical cancer? We do not have the answer but perhaps the next correspondent for a Commentary in 1991 will be able to write with greater certainty.

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Human papillomavirus in paired normal and abnormal cervical biopsies—implications for treatment

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Summary. A total of 143 consecutive patients with abnormal cervical cytology was examined at a large colposcopy clinic in Glasgow. Each patient had paired biopsies from normal and abnormal cervical epithelium examined both histologically and by immunoperoxidase staining for human papillomavirus (HPV) infection. More than 71% of the abnormal biopsies and 39% of the normal paired biopsies had histological evidence of HPV infection. Cytological evidence of HPV infection was seen in 38·5% of cervical smears. Immunocytochemistry revealed HPV antigen in 22% of the abnormal biopsies and in 4·2% of the 'normal' biopsies. The presence of HPV infection in colposcopically normal cervical tissue both inside and outside the transformation zone may help to explain why current methods for treatment of cervical HPV infection are often unsuccessful.

Several authors have reported a strong association between human papillomavirus (HPV) infection of the cervix and cervical neoplasia (Singer *et al.* 1985; zur Hausen 1977; Lancaster *et al.* 1986; Baird 1983; Syrjanen *et al.* 1985). HPV-16 DNA has been found in up to 90% of invasive cervical cancers (Schneider *et al.* 1985) and in 66% of patients with pre-invasive disease (McCance *et al.* 1985). Viral antigen indicating infection has been identified in the cervical tissue of 30% of patients with cervical intraepithelial neoplasia (CIN) (Singer *et al.* 1985). This strong association does not necessarily prove a causal role for HPV but recently Mitchell *et al.* (1986) in Melbourne have found a greatly increased risk of CIN in young women previously known to have had cytological evidence of HPV infection.

The marked increase in the incidence of cervical cancer and pre-cancer in women under 35 years of age and the increased frequency of HPV-associated cytological and histological abnormalities in this younger age group provide further evidence that eradication of HPV infection from the cervix should be attempted (Das & Duguid 1985; Wolfendale *et al.* 1983).

CIN can be effectively treated by surgery, electrocoagulation diathermy, carbon dioxide laser, cryocautery and cold coagulation (Hollyock & Chanen 1976; Carter *et al.* 1978; Duncan 1982; Richart *et al.* 1980) but viral lesions prove much more difficult to eradicate.

The possible reasons for this include reinfection from a sexual partner and multifocal genital tract lesions. However, when these are excluded there remains a substantial group of patients in whom one would have expected eradication of the viral lesion when this does not occur (Ferenczy *et al.* 1985). It seemed probable that in these women latent virus infection was present in genital tissue which looked apparently normal and was therefore not subjected to treatment

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when the atypical transformation zone was ablated.

To investigate this hypothesis we examined 143 patients attending a large city colposcopy clinic because of abnormal cytology. Biopsies were taken from colposcopically abnormal and adjacent normal cervical tissue and submitted for both histological examination and immunocytochemical analysis of HPV antigen.

A small subset of 29 patients had Southern blot hybridization studies carried out to verify the presence of HPV DNA sequences (Murdoch *et al.* 1988).

Patients and methods

A total of 143 patients attending a colposcopy clinic because of abnormal cytology had colposcopically directed punch biopsies taken from abnormal and normal cervical tissue. Cervical cytology was investigated in each patient before the procedure. The cervical biopsies were examined histologically. An indirect peroxidase antiperoxidase technique was set up to identify HPV antigen on the surface of cervical epithelial cells. Group-specific bovine papillomavirus (BPV) antiserum prepared in rabbits inoculated with disrupted purified BPV was used initially but more specific staining was subsequently obtained by using a commercial source of group-specific BPV antiserum (DAKO). This antiserum contains antibodies to disrupted virion components, i.e. to structural genes coded by the virus and highly conserved in HPV and BPV.

Non-stained paraffin specimens of punch biopsies were dewaxed in xylene and dehydrated in serial alcohol dilutions. Endogenous peroxidase was quenched with 0·5% hydrogen peroxide in methanol. The sections were then incubated in normal bovine serum to reduce non-specific background staining.

BPV antiserum was added in varying dilutions from 1/100 to 1/1000 and the sections were incubated overnight at 4 °C. Bridging antibody (swine anti-rabbit IgG) was applied for 30 min followed by the addition of peroxidase anti-peroxidase complex at 1:20 dilution for 10 min. The peroxidase reaction was developed with diamino benzoic acid (DAB) giving a brown precipitate in tissues containing papillomavirus antigen.

Haematoxylin was used as a counterstain. Each test was controlled by (i) omission of the BPV antiserum, (ii) use of HSV 2 antiserum instead of BPV antiserum, and (iii) use of normal rabbit serum to replace BPV antiserum.

Results

Histology

The histological criteria (Meisels *et al.* 1977) for diagnosis of HPV infection were acanthosis, accentuated rete pegs, cytoplasmic clearing (koilocytosis), multinucleation and dyskeratosis. Some or all of these histological features were seen in 102 of 143 (71%) abnormal cervical biopsies and in 55 of 143 (38%) paired normal biopsies of cervical tissue.

Cytology

The cytological criteria for HPV infection included dyskeratosis, binucleation, condensed hyperchromatic nuclei and koilocytosis (Meisels & Fortin 1976). This was reported in 72 of 143 patients (50%) (Table 1).

Immunocytochemistry

Dark brown nuclear staining of HPV antigen was mainly apparent in surface cells showing

Table 1. Evidence for human papillomavirus (HPV) infection in 143 'abnormal' biopsies

Abnormal biopsy	Total no	HPV histology		HPV cytology		HPV antigen	
		n	(%)	*n*	(%)	*n*	(%)
CIN III	54	29	(54)	25	(46)	7	(13)
CIN II	36	29	(81)	21	(58)	13	(36)
CIN I	22	18	(82)	10	(45)	5	(23)
Virus alone	26	26	(100)	15	(58)	6	(23)
Non-specific	5			1	(20)		
Total	143	102	(71)	72	(50)	31	(22)

koilocytotic atypia. Not all such cells gave positive staining and sections were therefore examined at several levels where possible

In 31 of 143 (22%) abnormal cervical biopsies HPV antigen was detected (Table 1). The frequency was lowest in patients with major degrees of CIN, particularly CIN III (13%), and this is in keeping with results from other geographical areas (Walker *et al.* 1983; Kurman *et al.* 1981). We examined seven invasive tumours at several levels and found evidence of HPV antigen in only one. In conclusion we found that the more malignant and de-differentiated the cell the less likely it is to show antigen expression.

In six of these 31 patients with HPV antigen in abnormal cells, HPV was also detected in the paired normal biopsy—showing that HPV virions (i.e. its infective form) can exist in apparently normal tissue. The overall rate in the study was 4%.

Discussion

The finding that almost three-quarters of our colposcopy patients have histological evidence of HPV infection will not come as a surprise to the colposcopist. It is interesting to note, however, that considerably fewer patients have HPV diagnosed on cytology smears. This would suggest a reservoir of HPV infection in the general population with apparently normal smears (Meanwell *et al.* 1987; Macnab *et al.* 1986).

If the prevalence of HPV infection in the normal population is similar to that seen in the patients with CIN then perhaps we must reconsider the theory that HPV is a causative agent in cervical neoplasia.

Immunoperoxidase staining appears to be a highly specific but rather insensitive test for the presence of HPV antigen in the tissues. Other workers have found a similar frequency of HPV antigen staining in CIN (Walker *et al.* 1983; Kurman *et al.* 1981). Molecular studies on a small sample (29 patients) of our patient group showed HPV-16 DNA in 58% of the abnormal and in 44% of the normal biopsies (Murdoch *et al.* 1988).

Immunocytochemical analysis, though appearing much less sensitive than DNA analysis for detecting HPV, does in fact detect capsid antigen produced in HPV infection and therefore detects the potentially infectious viral particle. Much HPV DNA does not appear to be transcribed in many neoplastic lesions and therefore could not be detected by a technique designed to detect capsid antigen—a product of intact replicating virus.

The real value of immunoperoxidase staining is its ability to detect potentially infectious virus. We have demonstrated that replicating virus is found in all stages of CIN and in invasive cancer. This may suggest that cofactors other than the differentiation of the cell are involved in the expression of HPV in tumours, and its ability to replicate.

The finding of HPV antigen in apparently normal cervical tissue outside the atypical transformation zone (ATZ) is highly significant clinically and explains why many patients after adequate local ablation to the ATZ return with persistent HPV infection of the cervix (personal observation).

Ferenczy *et al.* (1985) observed that most anogenital lesions recur close to the previously treated area and have suggested that extending the field of therapy might reduce the risk of recurrence. This theory is attractive. However, we are not in a position to define how far these margins should be extended, particularly in the case of cervix and vaginal fornices.

In relatively widespread disease the use of systemic or topical therapy, perhaps in combination with laser ablation, is logical but no treatment that is both safe and effective has yet been found.

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Factors associated with progression of cervical human papillomavirus (HPV) infections into carcinoma *in situ* during a long-term prospective follow-up

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Summary. In the course of a prospective study of 508 women with papillomavirus (HPV) lesions of the uterine cervix, 66 lesions that progressed into carcinoma *in situ* (CIS) were identified and treated by conization during a mean follow-up period of 35 months. The lesions were investigated with light microscopy and with in-situ DNA hybridization using ^{35}S-labelled probes for HPV 6, 11, 16, 18, 31 and 33. After radical cone treatment, 11 of the 66 women (16·7%) have presented with a recurrent HPV infection. The recurrence rate increased with the duration of the follow-up period from <10% at the mean follow-up of 25 months to 16·7% at the most recent follow-up at 35 months. Most of these 66 HPV lesions (89%) presented with concomitant CIN in the first punch biopsy, but it is noteworthy that the other 11% presented without concomitant CIN. HPV DNA of at least one of the six types examined was found in 73% of the first biopsies and it is noteworthy that the so-called 'low-risk' types, HPV 6 and 11, were found as frequently as the 'high-risk' types, HPV 16 and 18 (18% and 17%, respectively). This would suggest a similarity in the biological behaviour of these two HPV groups. Although the concept of the 'high-risk' and 'low-risk' HPV types may remain at least partially valid, it is imperative to realize that infection by HPV 6 and 11 by no means excludes the possibility for clinical progession into CIS and eventually to an invasive carcinoma.

Recent data have provided strong evidence to implicate human papillomavirus (HPV) in the aetiology of cervical intraepithelial neoplasia (CIN) and cervical cancer (Meisels *et al.* 1976; zur Hausen *et al.* 1984; Syrjänen 1984, 1986; Macnab *et al.* 1986; Mitchell *et al.* 1986). These

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data include the frequent coexistence of HPV and CIN in punch biopsies and Papanicolaou (PAP) smears, reports on malignant transformation of certain HPV lesions, and epidemiological evidence that both HPV infections and cervical cancer are sexually transmitted diseases (STD) (Meisels *et al.* 1976; zur Hausen et al. 1984; Syrjänen 1983; Syrjänen *et al.* 1984*a*; Oriel 1981; Kadish *et al.* 1986).

HPV structural proteins can be demonstrated immunohistochemically in CIN lesions, and the genome of one of the genital HPV types 6, 11, 16, 18, 31, 33, and the newly detected ones can be demonstrated in genital condylomas, CIN, CIS (carcinoma *in situ*), and in invasive carcinomas (Boshart *et al.* 1984; Dürst *et al.* 1983; Gissmann *et al.* 1984; McCance *et al.* 1985; Syrjänen *et al.* 1985*c,d*; Kadish *et al.* 1986; Kulski *et al.* 1987). Further supporting evidence has been provided by the elucidation of the molecular mechanisms involved, for instance in cell transformation by papillomaviruses (Broker & Botchan 1986).

To investigate the biological behaviour of cervical HPV infections unaffected by any treatment, a prospective follow-up study of such women has been conducted in our clinic since 1981 (Syrjänen *et al.* 1984*b*, 1985*a,b,c*, 1987*a,b*). Our follow-up results suggest that the natural history of cervical HPV infections is similar to that of CIN lesions (Syrjänen *et al.* 1985*a,b,c,d*) which, if untreated, can progress into a frankly invasive cervical cancer (Koss *et al.* 1963; Nasiell *et al.* 1986). Our recent data also suggested that HPV type might be an important determinant in the natural history of cervical HPV lesions (Syrjänen *et al.* 1985*c*), thus supporting the concept of low-risk (HPV 6 and 11) and high-risk (HPV 16 and 18) types in cervical carcinogenesis (Boshart *et al.* 1984; zur Hausen *et al.* 1984; Syrjänen *et al.* 1985*d*; Kulski *et al.* 1987).

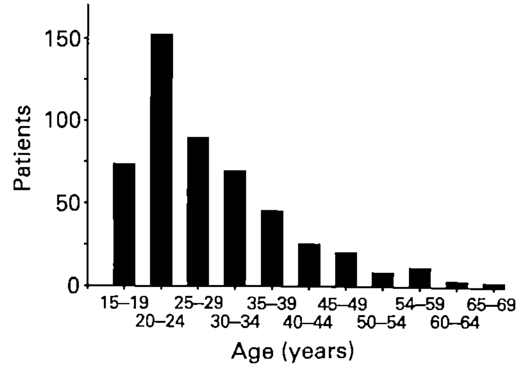

Fig. 1. Age distribution of the 508 women at the time of recruitment in the follow-up study (mean age 28·7, SD 10·2 years).

To date, more than 500 women with cervical HPV infections have been followed prospectively for a mean of 35 (SD 19) months (Syrjanen *et al.* 1987*a,b*). Although the figures for regression, persistence and progression have remained relatively stable since the 18-month follow-up, the number of HPV lesions progressing to CIS (and treated by conization) has steadily increased (Syrjänen *et al.* 1984*b*, 1985*a,b,c*, 1987*a,b*). Because these lesions represent the immediate precursors of an invasive cervical cancer (Koss *et al.* 1963; Nasiell *et al.* 1986), the data obtained from lesions that progressed to CIS were analysed further. The aim was to investigate the factors that could predict such an aggressive clinical course and thus provide information for treating these HPV infections associated with a definite malignant potential.

Patients and methods

Study outline

The present series consisted of 508 consecutive women currently included in a prospective follow-up study of cervical HPV infections conducted in our clinic since 1981 and described previously (Syrjänen *et al.* 1984*b*, 1985*a,b,c*). The women were invited to participate in the study whenever HPV-induced cytological changes were found in their routine PAP smears (Meisels *et al.* 1976; Syrjänen 1983, 1984, 1986). The patients were divided initially into two groups on the basis of concomitant changes suggestive of CIN seen in the first PAP smear: women with concomitant CIN were categorized to group A and those without CIN were categorized to group B, as described previously (Syrjänen *et al.* 1985*a,b,c*). The women were examined at the outpatient department of gynaecology, Kuopio University Central Hospital, at 6-month intervals.

The age distribution of the 508 patients is shown in Fig. 1; the mean age was 28·7 (SD 10·2) years, the mode was between 20 and 24 years. The distribution by the length of follow-up is given in Fig. 2; the mean follow-up time was 34·6 (SD 18·9) months.

At each attendance, the women had a thorough gynaecological examination, including colposcopy and PAP smears with or without

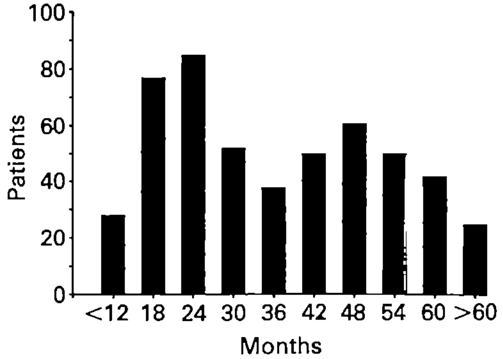

Fig. 2. Distribution of the 508 women according to the extent of the follow-up (mean follow-up 34·6, SD 18·9 months).

directed punch biopsy (groups A and B, respectively). It is to be emphasized that the lesions were eradicated by conization whenever progression into carcinoma *in situ* (CIS) was diagnosed (Syrjänen *et al.* 1985*a*). The present report specifically analyses the findings in 66 women in whom the HPV lesions progressed into CIS during the follow-up period.

The clinical course of each HPV lesion was classified as either regressed, persistent, progressed, or recurred, depending on the presence or absence of (i) the lesion on colposcopy, (ii) HPV-induced cytological changes in PAP smears, and (iii) typical morphology in punch biopsies (Syrjänen *et al.* 1985*a,b,c,* 1987*a*). A lesion was classified as regressed when all three tests were negative. A positive finding in any one of the three tests categorized the lesion as persistent. Clinical progression was always diagnosed by a punch biopsy, showing a change from HPV without CIN to HPV with concomitant CIN (HPV-CIN) or from lower to higher grade of HPV-CIN.

Cervical punch biopsy

In haemotoxylin and eosin stained sections, morphology of the HPV lesions was classified as flat, inverted or papillary condyloma using the criteria described previously (Meisels *et al.* 1976; Syrjänen 1983, 1984). The grade of CIN was assessed using the commonly accepted criteria for CIN I, II and III (Koss *et al.* 1963; Nasiell *et al.* 1986).

Although the current definition of CIN III includes CIS, we use CIS in this series to describe a subcategory of CIN III to differentiate it from severe dysplasia.

Demonstration of HPV structural proteins

An indirect immunoperoxidase (IP-PAP) staining for HPV structural proteins was completed in paraffin-embedded sections, as described previously by Syrjänen (1983). Commercially available IP-PAP kits (DAKOPATTS, Glostrup, Denmark) were used, the antiserum being raised against the papillomavirus-specific structural antigens.

Detection of HPV DNA

For HPV typing, the specific DNA probes for HPV types 6, 11, 16, 18, 31 and 33 were used in the present study. The in-situ DNA hybridization technique applied to routine paraffin sections was performed as described by Gupta *et al.* (1985) with some modifications, using the ^{35}S-labelled DNA probes under stringent conditions (50% formamide, $T_m - 20°C$), as described previously (Syrjänen *et al.* 1986*a,b*). The localization of HPV DNA is indicated by the dense condensations of black silver grains after autoradiography, superimposed usually on nuclei of the intermediate and superficial cells in HPV lesions (Fig. 3).

Results

The current follow-up results of the 508 women are summarized in Table 1. In 29% the HPV infection regressed spontaneously, in 56% the lesion persisted and in 14% the lesions showed clinical progression. Of the 508 women in the series, 66 (12·9%) had lesions that progressed into CIS, and they were therefore treated by conization; so far 11 (16·7%) of them have presented with a new HPV lesion at one of the subsequent follow-up visits and they have been classified as recurrent infections.

The rate of recurrences after conization in these 66 women increased with the follow-up time, the rate was <10% at a mean follow-up of 25 months and had increased to 16·7% at the follow-up 1 year later (35-month follow-up).

The histological grade of these 66 HPV lesions at the first punch biopsy assessment is shown in Table 2. Most lesions (48·5%) were HPV-CIN III at the time of the first clinic visit. Such patients were always subjected to cone treatment within less than 2 months after entry to the study, but 10·6% of the lesions showed no concomitant CIN when first seen at the clinic.

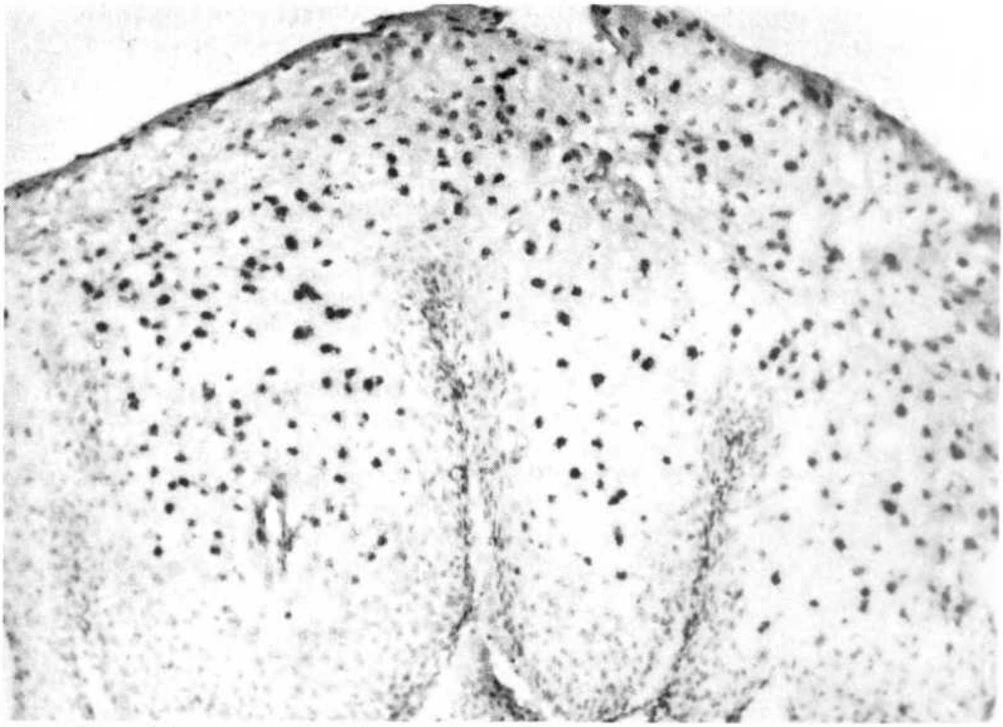

Fig. 3. A flat condyloma processed for demonstration of HPV 11 DNA by in-situ hybridization under stringent conditions. Black silver grains after autoradiography indicating the localization of HPV 11 DNA sequences are found superimposed on the nuclei of intermediate cells. (In-situ hybridization with ^{35}S-labelled HPV 11 DNA probe, original magnification ×100.)

The HPV typing data of the first punch biopsies of the 66 lesions that progressed to CIS are summarized in Table 3. HPV DNA of at least one of the six types investigated was found in 72·6% of cases; double infections (in any possible combinations) were found most frequently. It is noteworthy that the so-called low-risk types, HPV 6 and 11, were present as often as the high-risk types, HPV 16 and 18 (18·1% and 16·6% respectively).

Discussion

Since 1981, more than 500 women infected with HPV in the uterine cervix have been followed prospectively in our clinic to investigate the natural history of this infection, unaffected by therapeutic interventions (Syrjänen *et al.* 1987*a,b*). After a short (<1 year) follow-up of a limited number of women, we found regression in 47%, persistence in 47%, and progression in the other 6% (Syrjänen *et al.* 1984*b*). After a mean follow-up of 18 months in 343 women, the corresponding figures were 25%, 61% and 14%, respectively, and 14 (4%) had progressed into CIS (Syrjänen *et al.* 1985*a,b*). These data are in complete agreement with those reported by de Brux *et al.* (1983) from a retrospective study of 764 cervical HPV lesions followed for 18 months; 27% regressed, 53% persisted, and 20% progressed. In our study, after a mean follow-up of 20 months in 418 women, the figures for regression, persistence, and progression remained relatively stable at 24%, 55%, and

Table 1. Clinical course of cervical human papillomavirus infections in 508 women during a mean follow-up of 35 months

Clinical course	Number of women (n = 508) n (%)
Regressed	145 (28·6)
Persisted	282 (55·6)
Progressed	69 (13·6)
Recurred	11 (2·2)
Cone treated	66 (12·9)

Table 2. Histological grade at first punch biopsy of 66 cervical human papillomavirus (HPV) lesions that progressed into carcinoma *in situ*

Histological grade	*n* (%)
HPV-CIN III	32 (48·5)
HPV-CIN II	14 (21·2)
HPV-CIN I	13 (19·7)
HPV only	7 (10·6)
Total	66

CIN, Cervical intraepithelial neoplasia.

21%, respectively. However, the proportion of lesions progressing to CIS had increased to 11% (44 women) and after the mean follow-up period of 25·6 months, in 1986, it had increased to 12% (Syrjänen *et al.* 1985*c*, 1987*b*).

The present series is the largest with the longest period of prospective follow-up of women with cervical HPV infections. Indeed, there are only a few prospective follow-up studies of women with cervical HPV infection (Evans & Monaghan 1985; Minucci *et al.* 1986; Nash *et al.* 1987), although the natural history of classical CIN lesions has been investigated in great detail (Koss *et al.* 1963; Nasiell *et al.* 1986). The clinical course described for CIN is similar to our findings for HPV infections (Table 1).

The reliability of such studies improves with the follow-up time (Nasiell *et al.* 1986; Syrjänen 1987*a,b*) as is clearly demonstrated by our findings that the figures for regression, persistence, and progression stabilized only after a remarkably long follow-up time of 18 months (Syrjänen *et al.* 1985*a,b*). Thus, the present long-term follow-up results differ markedly from those based on a short-term follow-up of a limited series of women reported by Evans & Monaghan (1985), Minucci *et al.* (1986) and Nash *et al.* (1987) but their figures closely parallel our short-term results (Syrjänen *et al.* 1984).

The object of the present study was to investigate the factors associated with clinical progression into CIS, because we had observed a steadily increasing number of such progressions from 4% at 18-month follow-up to 13% at present. In parallel, the number of HPV lesions that recurred after radical cone treatment was increased from 0·4% to 2·2% in the whole series of 508 women during that time and specifically from 10 to 17% in 1 year in the 66 women detailed in the present study. There was a significant correlation between recurrence and the presence of CIN in the first biopsy; none of the HPV lesions without CIN in the first biopsy has recurred so far, even if the lesion progressed to CIS.

Indeed, the clinical course of HPV lesions has been shown to be markedly influenced by the grade observed in the first biopsy (Syrjänen *et al.* 1985*a*, 1987*a*; de Brux *et al.* 1983; Evans & Monaghan 1985; Minucci *et al.* 1986). The rate of progression was significantly higher for lesions with CIN than for those without CIN; the latter showed a significantly higher rate of spontaneous regression (Syrjänen *et al.* 1987*a,b*). Surprisingly then, 11% of the lesions that progressed to CIS were without concomitant CIN in the first biopsy (Table 2). Although most of the lesions that progressed to CIS had concomitant CIN in the first biopsy, this was not essential for progression to occur. This is an important factor in the histopathological assessment of HPV infections (Syrjänen 1986).

HPV type cannot be predicted from the light microscopic appearance of the lesions (Kadish *et al.* 1986; Syrjänen *et al.* 1987*a*). Most studies have found HPV 6 and 11 confined to benign lesions (condylomas and CIN I) and HPV 16 and 18 associated with severe dysplasia, CIS and invasive carcinomas (Boshart *et al.* 1984; Dürst *et al.* 1983; Gissmann *et al.* 1984; McCance *et al.* 1985; Kadish *et al.* 1986; Kulski *et al.* 1987). This suggests that there are 'low-risk' and 'high-risk' HPV types in cervical carcinogenesis (zur Hausen *et al.* 1984). In general, a single biopsy from each lesion was analysed in these studies, with no available follow-up data on the previous clinical course of the lesion. Recently, we documented a lesion that progressed from CIN I to an

Table 3. Human papillomavirus (HPV) types demonstrated by in-situ DNA hybridization in the first punch biopsy of 66 cervical HPV infections that progressed into carcinoma *in situ*

Type	*n* (%)
HPV-negative	18 (27·4)
Double infection	13 (19·7)
Single infection	
HPV-6	5 (7·5)
HPV-11	7 (10·6)
HPV-16	7 (10·6)
HPV-18	4 (6·0)
HPV-31	5 (7·5)
HPV-33	7 (10·6)

invasive cancer in under 3 years, and which showed a double infection with HPV 16 and HPV 18 (Syrjänen *et al.* 1985*d*). The present project is the first where data on the influence of the HPV type on the clinical course of HPV infections are available.

Syrjänen *et al.* (1985*c*) reported the highest progression rate in association with lesions of HPV 16 (33·3%), which also had the lowest regression rate (5·6%) in contrast to the regression rate of 26% in lesions induced by HPV 6 or 11 (Syrjanen *et al.* 1987*a,b*). However, these HPV 6 and HPV 11 lésions had a relatively high (25·6%) progression rate which suggests that these 'low-risk' types HPV 6 and 11 also represent an increased risk for CIS. Mitchell *et al.* (1986) calculated that the relative risk to develop CIS was 15·6 for all women with HPV, and 38·7 for those under 25 years of age. The life-table analysis of our present follow-up data indicates, however, that progression into CIS required significantly longer time than progression into the less severe grades of CIN (data not shown in the present communication).

Using the sensitive in-situ DNA hybridization technique and HPV probes 6, 11, 16, 18, 31 and 33 (Syrjänen *et al.* 1986*b*), HPV DNA was identified in 72·6% of the lesions that progressed to CIS. Double infections comprised the bulk (19·7%) of HPV infections, followed by HPV 11, 16 and 33 with a 10·6% frequency each. Noteworthy again is the fact that the low-risk types, HPV 6 and 11, were involved in 18·1% of the lesions that progressed into CIS. These data are in full agreement with our previous observations on the clinical progression of HPV 6 and 11 infected lesions (Syrjänen *et al.* 1987*b*). These findings are supported by the observations of others (Kulski *et al.* 1987), who have found HPV 6 sequences also in invasive cervical carcinomas. On the other hand, HPV 16 DNA has been recently demonstrated in clinically normal cervical squamous epithelium (Macnab *et al.* 1986).

Although the concept of high- and low-risk HPV types may still be valid, in that most malignant lesions are associated with high-risk types, our findings have shown a similarity in the biological behaviour of these HPV groups. It is important to realize that infection by HPV 6 and 11 by no means excludes the possibility for clinical progression into CIS and eventually to an invasive carcinoma.

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Significance of smoking and detection of serum antibodies to cytomegalovirus in cervical dysplasia

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Summary. Cervical biopsy specimens from 422 women attending a colposcopy clinic showed various grades of dysplasia in 387 and no evidence of dysplasia in 35. Of the women with dysplasia (CIN I, II or III) 67% were smokers and 33% were non-smokers compared with 43% smokers and 57% non-smokers in those without evidence of dysplasia. Of the women with CIN I 56% were smokers and 44% were non-smokers; 66% of those with CIN II were smokers and 34% were non-smokers ($P<0{\cdot}02$), and 71% of those with CIN III were smokers ($P<0{\cdot}01$). There were no significant differences between the smokers and non-smokers in the proportion of women who had had either a miscarriage or an abortion, in the prevalence of condylomata acuminata, and the use of oral contraception or barrier methods. Neither were there any significant differences in numbers of patients seropositive for cytomegalovirus or in CMV antibody titres among the groups. The detection of koilocytic cells in cervical biopsies showed a significant difference between smoking and non-smoking groups which reflected a significant increase in koilocytosis in smokers with CIN III.

Data from the Third National Cancer Survey analysed by Winkelstein *et al.* (1977) revealed a high correlation between the incidence rates for lung cancer in men and cervical cancer in women. Cigarette smoking was most strongly associated with squamous cell carcinomas such as of the lung, larynx or oesophagus, and it was proposed that the common factor linking lung and cervical cancers may be the histological type (Winkelstein 1977). A variety of studies have since taken several potentially confounding factors into account, and an association between smoking and cancer of the cervix persisted (Wright *et al.* 1978; Harris *et al.* 1980; Buckley *et al.* 1981; Lyon *et al.* 1983; Trevethan *et al.* 1983). However, some unknown risk factor may be confounding (Wright *et al.* 1978).

Three viruses have been implicated in the aetiology of cervical intraepithelial neoplasia (CIN); namely, herpes simplex virus type 2 (HSV-2), cytomegalovirus (CMV) and, most recently, human papillomavirus (HPV) (reviewed by Zur Hausen 1982; Baird 1985). Evidence to support each of these candidates is not definitive and it seems likely that several co-factors may be involved. In the present study we investigated the prevalence and quantity of smoking amongst patients with various degrees of CIN and a control group. Age, contraception, pregnancy, abortion, miscarriage, and the presence of condylomata acuminata were considered with respect to smoking habits. Where possible, serum samples were obtained and antibody titres to CMV estimated. Koilocytic cells found in sections of cervical biopsies and considered

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pathogonomic for HPV were noted. These variables were analysed in relation to smoking and CIN.

Patients and methods

The study involved groups of women attending the Lothian Area colposcopy clinic. Colposcopically directed punch biopsies were taken from up to four areas of the cervix, depending on the extent of abnormality seen, and examined using routine histopathological methods on paraffin sections. Seventy patients were classified as having mild dysplasia (CIN I), 83 had moderate dysplasia (CIN II) and 234 had severe dysplasia/carcinoma *in situ* (CIN III). If a woman had more than one cervical biopsy, she was classified by the biopsy report with the most severe lesion. The presence or absence of koilocytes in sections was also noted. The healthy controls comprised 35 patients. These had been referred to the clinic with normal or mildly abnormal (not more than class 1+) cervical smears. Some of the normal group had smears showing inflammatory changes. Punch biopsies showed no evidence of CIN.

The patients were asked if they had smoked in the past year, and, if so, how many cigarettes per day. Any person smoking more than five per day was categorized a 'smoker'. Ex-smokers were categorized as 'non-smokers'. On the case notes were recorded contraceptive method where used, pregnancies, miscarriages and abortions, and any condylomata acuminata. Women who were pregnant within 1 year of the cervical biopsy were excluded from the study.

Chi-squared tests were used to detect differences between smokers and non-smokers with respect to grade of dysplasia, age, pregnancy, rates of abortion or miscarriage, contraceptive method, condylomata acuminata, and the presence of koilocytes in cervical biopsies.

Blood samples were obtained at the time of cervical biopsy from 181 of the patients. Serum was separated and stored at −20 °C.

Detection of antibodies to CMV by enzyme-linked immunosorbent assay (ELISA)

CMV-cell antigen preparation. Human embryonic fibroblasts (HEF) at pass 3 to 10 were grown in modified Eagle's medium supplemented with 5% fetal calf serum (FCS), 100 i.u./ml penicillin and 200 μg/ml streptomycin. When confluent, some cells were infected with CMV, strain AD-169, at one to two plaque-forming units per cell and maintained in medium containing 2% FCS at 37 °C until advanced cytopathic effects (CPE) were observed in the infected cultures. The monolayers were washed with cold phosphate-buffered saline (PBS) and harvested using glass beads. Cells (5×10^6) were washed three times in PBS and resuspended in 1 ml glycine buffer (five parts 0·1 M glycine plus three parts 0·1 M NaOH, prepared on day of use). They were ultrasonicated for 2 min at 9 kc/s and stored at −70 °C until required. Protein concentrations were estimated by the method of Lowry.

ELISA. The procedure was a modification of the method described by Bidwell *et al.* (1977). The assay was performed on polystyrene flat-bottomed microtitre plates (Nunc). Wells were coated with 50 μl of CMV-infected cell antigen or uninfected cell antigen (diluted from 400 to 1000 times) in 0·05 M carbonate buffer, pH 9·6, overnight at 4 °C. Plates were washed three times with 0·05 M PBS, pH 7·2, containing 0·1% bovine serum albumin (BSA), 0·05% Tween 20. Each serum was diluted from 1/100 to 1/12 800 in 0·05 M PBS, pH 7·2, containing 1% BSA, 0·05% Tween 20 and tested against infected-cell antigen and control antigen. Fifty microlitres of diluted serum were added to each well and the plates were incubated at room temperature for 6–8 h. After washing again, 50 μl of sheep anti-human IgG conjugated with horseradish peroxidase (Scottish Antibody Production Unit, diluted in 0·05 M PBS, pH 7·2, containing 1% BSA, 0·05% Tween 20) were added and the plates were incubated overnight at 4 °C. Washing was followed by the addition of substrate, 50 μl of 0·4 mg/ml *o*-phenylene-diamine (Sigma) in 0·15 M phosphate, 0·027 M citrate buffer, pH 6·0, containing 0·2 μl/ml (v/v) 30% H_2O_2. The reaction was stopped by addition of 100 μl 4 M H_2SO_4. Absorbance at 490 nm was measured with a multiscan (Titertek, Flow). In each experiment, negative and positive controls were included: sera from healthy individuals known to have sufficiently low and high antibody titres to CMV.

On each plate, eight wells contained serum (diluted 1/100) which was negative for CMV. A positive reaction was denoted by absorbances >3 SD above the mean of the negative serum. Positive reactions to uninfected cell antigen did not occur.

Chi-squared tests were used to determine differences in the numbers of smokers: non-smokers with antibodies to CMV for each stage of cervical dysplasia.

The Kruskal–Wallis analysis of variance was used to determine differences in antibody titres to CMV among the patients.

Results

Numbers of smokers and non-smokers with various grades of CIN

In our study group of 422 womèn, 148 were non-smokers and 274 were smokers (Table 1). Of the 35 women with no evidence of dysplasia 57% were non-smokers and 43% were smokers, and of the 70 with CIN I 44% were non-smokers and 56% were smokers. In the women with CIN II and III, the ratio of non-smokers to smokers changed, only 34% of those with CIN II and 30% of those with CIN III were non-smokers. When the numbers of non-smokers: smokers in each stage were compared with the controls (no dysplasia) by χ^2-test, statistically significant differences were seen for stage CIN II ($P<0·02$) and stage CIN III ($P<0·01$). Of the 274 smokers, 60% had stage CIN III disease; of the 148 non-smokers 47% had CIN III ($P<0·01$). The age range of patients in each group was similar as was the mean age.

Table 1. Numbers and age ranges of smokers and non-smokers with various grades of cervical histology

	Cervical histology			
Smoking category	Normal	CIN I	CIN II	CIN III
Non-smokers				
No. of women	20	31	28	69
Age (years)				
Mean	33·6	29·5	32·6	32·5
Range	19–50	17–46	20–48	15–59
Smokers				
No. of women	15	39	55	165
Age (years)				
Mean	29·7	33·1	28·6	31·1
Range	20–46	22–49	19–46	20–60
Total no.	35	70	83	234
% non-smokers	57·1	44·3	33·7	29·5
% smokers	42·9	55·7	66·3*	70·5†

Significance of difference in percentage of smokers compared with normal histology group * $P<0·02$, † $P<0·01$.

Table 2. Numbers of smokers and non-smokers and grade of cervical histology

			Cervical histology							
Smoking category	Age (years)	n	Normal		CIN I		CIN II		CIN III	
Non-smokers	18–25	27	2	(7·4%)	10	(37%)	4	(14·8%)	11	(40·7%)
	26–33	63	7	(11·1%)	12	(19%)	12	(19%)	32	(50·8%)
	34–41	42	8	(19%)	6*	(14·3%)	9	(21·4%)	19†	(45·2%)
	>41	16	3	(18·8%)	3	(18·8%)	3	(18·8%)	7	(43·8%)
Total		148	20		31		28		69	
Smokers	18–25	61	5	(8·2%)	5	(8·2%)	19	(31·1%)	32	(52·5%)
	26–33	129	7	(5·4%)	17	(13·2%)	27	(20·9%)	78	(60·5%)
	34–41	68	1	(1·5%)	13*	(19·1%)	7	(10·3%)	47†	(69·1%)
	>41	16	2	(12·5%)	4	(25%)	2	(12·5%)	8	(50%)
Total		274	15		59		55		165	

Significance of difference in percentage of smokers compared with normal histology group in the same age range (34–41) * $P<0·01$, † $P<0·001$.

Smokers and non-smokers in each category of dysplasia were further subdivided according to age (Table 2). The only significant differences between smokers and non-smokers occurred in the age group 34–41 years for women with CIN I ($P<0{\cdot}01$) and for those with CIN III ($P<0{\cdot}001$). Of the 42 non-smokers in this age group 19 (45%) had CIN III and of the 68 smokers in this age group 47 (69%) had CIN III.

Of the 234 women with CIN III 43 (18%) were under the age of 26 and 74% of them were smokers, 153 (65%) were under 34 years of age and 72% of them were smokers, and of the 219 women under 41 years of age 157 (72%) were smokers.

Obstetric history and contraception

Of the 422 women in the study 324 (76·8%) had been pregnant, 79·9% of the 274 smokers and 70·9% of the 148 non-smokers ($P<0{\cdot}05$). This difference reflected a significant increase in smokers with CIN I (Table 3); 34 of the 39 smokers with CIN I and 19 of the 31 non-smokers with CIN I ($P<0{\cdot}02$) had been pregnant. For each grade of dysplasia there was no significant difference in the proportion of women with a history of miscarriage or abortion between smokers and non-smokers.

Overall, 39·8% of the women were using oral contraceptives, 41·6% of the smokers and 36·5% of the non-smokers. There were no significant differences between smokers and non-smokers when individual categories of dysplasia were considered, and no differences within smoking and non-smoking groups. Overall, 9·7% of the women were using barrier contraceptives, 7·7% of the smokers and 13·5% of the non-smokers. There were no significant differences with grade of dysplasia between smokers and non-smokers, and no differences within smoking and non-smoking groups.

Prevalence of condylomata acuminata and koilocytosis

There were no significant differences in the prevalence of condylomata acuminata between smokers and non-smokers with grade of dysplasia and no differences within smoking and non-smoking groups.

Of the cervical biopsies with dysplasia which could be categorized on the basis of koilocytic cells being present, 85·6% demonstrated koilocytes. Significantly more smokers (93·5%) than non-smokers (71·6%) had koilocytic cells ($P<0{\cdot}001$). This difference reflected a significant increase in the prevalence of koilocytosis in smokers with CIN III (Table 4).

Numbers of cigarettes smoked

The mean number of cigarettes smoked by the healthy women per day was 15 (SD 3·8), this was similar to the mean number smoked by patients with CIN I, II or III.

Table 3. Frequency distribution by obstetric history, smoking category and grade of cervical histology

	Cervical histology			
Smoking category	Normal	CIN I	CIN II	CIN III
Non-smokers				
Total no.	20	31	28	69
Obstetric history (n)				
Ever pregnant	15	19*	23	48
Induced abortion	2	2	5	6
Miscarriage	2	6	2	8
Smokers				
Total no.	15	39	55	165
Obstetric history (n)				
Ever pregnant	10	34*	44	131
Induced abortion	3	9	7	23
Miscarriage	2	9	10	22

Significance of difference between smokers and non-smokers * $P<0{\cdot}02$.

Table 4. Numbers of smokers and non-smokers with evidence of koilocytes in cervical biopsies

Smoking category	Cervical histology CIN I	 CIN II	 CIN III
Non-smokers			
Koilocytes			
Present	20	16	32*
Absent	4	5	18
Not classified	7	7	19
Smokers			
Koilocytes			
Present	34	33	91*
Absent	1	5	5
Not classified	4	17	49

* Significance of difference between smokers and non-smokers $P<0{\cdot}001$.

Antibodies to CMV

Overall, 81·8% of the women had antibodies to CMV, 79·8% of the non-smokers and 82·8% of the smokers. For each grade of dysplasia there were no significant differences in the proportion of women who were seropositive between smokers and non-smokers. There were no significant differences in CMV antibody titres between smokers alone, between non-smokers alone or between smokers and non-smokers (Table 5).

When the patients were considered in age groups, more smokers than non-smokers had antibodies to CMV, but this was not statistically significant. There were too few patients to compare smokers and non-smokers in the age groups 18–25 and >41 years. There was a general trend of increasing numbers of patients seropositive for CMV with age.

Discussion

In our study group of 422 women, more smokers than non-smokers presented with cervical dysplasia and this mainly reflected a significant increase in smokers with CIN III particularly in the 34–41 age group. There was also a significant increase in smokers with CIN II which suggests a progressive increase in the number of smokers with progression of dysplasia. The lack of difference with CIN I may simply reflect the minor differences in pathological staging which distinguish CIN I from normal histology. Trevethan *et al.* (1983) also found that cigarette smoking was more strongly associated with severe dysplasia and carcinoma *in situ* (CIS) than with milder dysplasia. Cumulative exposure to tobacco smoke was related to risk of dysplasia and CIS and they found relative risks for past smokers intermediate between those for current smokers and non-smokers. La Vecchia *et al.* (1986) reported that within 10 years of starting smoking there is an increase in risk of intraepithelial neoplasia, and suggest the effects of smoking are manifest at an early stage of cervical cancer, presumably CIN. Although we

Table 5. Cytomegalovirus (CMV) seropositivity and antibody titres in smokers and non-smokers according to grade of cervical histology

	Cervical histology							
	Normal		CIN I		CIN II		CIN III	
Titre	Non-S	S	Non-S	S	Non-S	S	Non-S	S
>1/100	1	1	2	4	1	6	8	10
<1/100	1	1		1			1	9
<1/200			1	1		1	2	10
<1/400		1	1		1	1	1	4
<1/800			1	1	1	2	2	2
<1/1600	1	1	2	1	1		4	3
<1/3200	2	3	2	3	1	5	3	10
<1/6400	1				1	1	4	12
<1/12 800	1	1	1	5	3	6	8	16
No. of patients	7	8	10	16	9	22	33	76
No. of patients seropositive	6	7	8	12	8	16	25	66

Non-S, Non-smoker; S, smoker. Sera were negative at a titre of >1/100 have not studied patients with invasive carcinoma and did not determine duration of exposure to cigarette smoking, the similarities in mean age and mean numbers of cigarettes consumed by the individual groups may also suggest that the effects of smoking occur early in the development of cervical cancer.

The alternative point of view is that the effects of cigarette smoking in cervical cancer are either fairly weak or are an artefact of other variables. Although we were unable to control for many potential confounding factors, we found no significant differences between smokers and non-smokers with respect to pregnancy, abortion, miscarriage, condylomata acuminata, oral contraception and the use of barrier methods. Some of these conditions may be considered indicators of sexual behaviour and of socio-economic class. However, duration of oral contraceptive use was not determined, and there may be an association between cervical cancer and long-term oral contraception (Ory *et al.* 1977; Peritz *et al.* 1977) Other factors which may be important are the husband's sexual behaviour, history of venereal disease and smoking habits (Skegg *et al.* 1982; Zunzunegui *et al.* 1986).

It is thought that a viral agent could be the initiator or a promoter of neoplastic development in the cervix. Cytomegalovirus has oncogenic potential *in vitro* (Clanton *et al.* 1983; Galloway *et al.* 1984; Nelson *et al.* 1984), and has been shown to induce cervical tumours in mice (Heggie *et al.* 1986). CMV has been found in cervical secretions and has been isolated from cell cultures of cervical biopsies from women with advanced carcinoma (Melnick *et al.* 1978). Vestergaard *et al.* (1972) reported a significant increase in serum antibodies to CMV in patients with newly diagnosed cervical cancer compared with matched controls, although several groups have failed to confirm this association (Fucilla *et al.* 1971; Pasca *et al.* 1975; Hart *et al.* 1982). The present study also found no significant differences in CMV seropositivity or antibody titres between women without cervical dysplasia and the women with various grades of cervical dysplasia. In addition, there were no significant differences in CMV seropositivity or antibody titres among controls and smokers and non-smokers with cervical dysplasia. CMV DNA sequences have been found in only a small number of histologically abnormal cervices (Huang *et al.* 1984; Fletcher *et al.* 1985), suggesting that presence and continued expression of the CMV genome are not essential to maintenance of the malignant state. Alternatively, if CMV itself is not responsible for neoplastic development in the cervix, neoplastic changes may predispose the cervix to infection or reactivate latent virus.

There is mounting evidence for an aetiological association between cervical cancer and human papillomavirus (HPV) infection. DNA from HPV-16 and HPV-18 has been found in cervical biopsies from patients with dysplasia and cancer of the cervix (Boshart *et al.* 1984; Crum *et al.* 1985; Lehn *et al.* 1985). Durst *et al.* (1983) reported integration of the HPV-16 genome into host cell DNA, and recently Shirasawa *et al.* (1986) have suggested that integration may occur in the precancerous stage. Although in our study more smokers than non-smokers showed evidence of condylomata acuminata this difference was not significant. Significantly more smokers had koilocytes in their cervical biopsies; this was most pronounced in the group with CIN III.

There are two main mechanisms for explaining an association between cigarette smoking and cervical cancer. Absorbed carcinogenic components of cigarette smoke may be transported in the blood to the cervix. Support for this hypothesis comes from the recent detection of nicotine and cotinine in the cervical fluid of smokers (Sasson *et al.* 1985). Alternatively, or in addition, tobacco smoking appears to lead to immune suppression. Although there are inconsistent findings as to the effects of smoking on cellular immune responses, suppression of serum immunoglobulins is well documented (Vos-Brat & Rumke 1969; Kosmider *et al.* 1973) and pronounced depression of antibody levels may occur at particular sites (Bahna *et al.* 1983). Such immune suppression could be responsible for reactivation of viruses remaining latent in cervical tissues, if not a herpesvirus, then perhaps HPV or another, as yet unidentified, virus.

In agreement with Vonka *et al.* (1984) we found increasing numbers of women in the younger age groups presenting with CIN III, which further emphasizes essential screening of young sexually active women. It is also evident that women with cervical dysplasia are more likely to be smokers and that smokers are more likely to have CIN III disease. The association of koilocytosis with dysplasia suggests the involvement of HPV and our results indicate that synergism with cigarette smoking is likely.

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Rapidly progressive cervical cancer: is it a real problem?

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Summary. The appearance, in younger women, of a rapidly progressive form of cervical carcinoma has been suggested. This, together with evident deficiencies in the provision and technique of screening services, has been taken to indicate a need for more frequent screening. We show that a substantial increase in the observed number of such rapidly progressive cancers would be expected simply on the basis of changes in population structure, a general increase in cervical cancer rates and increases in the proportion of women screened.

Despite the lack of randomized controlled trials there is now little doubt that well-organized screening programmes do reduce the incidence of invasive cervical carcinoma (Hakama *et al.* 1986). The issues of how often and at what ages to screen remain open, partly because health priorities inevitably vary with time and place. In particular, demands for increased screening of younger women (<35 years of age) are often made on the grounds that these women are at increasing risk of a rapidly progressive form of the disease with a short in-situ phase (Berkeley *et al.* 1980; Paterson *et al.* 1984; Bamford *et al.* 1983; Prendiville *et al.* 1980). It has been suggested that this is a new form of the disease distinct from the rapidly progressive form postulated by Ashley (1966) to occur in older women. During the last decade there has been increasing interest in human papillomavirus (HPV) infections of the cervix and their possible relevance to cervical cancer (Editorial 1982), although one study, at least, failed to support the idea that HPV infections may be related to the apparent increase in rapidly progressive cancers (Singer *et al.* 1985).

However, other changes have occurred all of which could affect the number of apparently fast growing cancers that are seen each year. In England and Wales the incidence rates of invasive cervical cancer in women <35 and in the 35–44 age group have increased during the period 1972–1982, whilst those in older women have fallen or remained stable (Table 1). These differences are consistent with a cohort effect (Beral 1974; Osmond *et al.* 1983) reflecting changes in the sexual habits of each generation. Despite the relatively inefficient organization of screening services in England and Wales the existing programme may in fact have prevented further increases in incidence and mortality (Cook & Draper 1984). Also, changes in the population structure have increased the number of women at risk in the younger age groups (Table 2). Again the extent of screening has altered. Estimates of the total number of smears per year (Roberts 1982) were 2124×10^3 in 1972 and 2928×10^3 in 1980, a 38% increase overall but more pronounced in women <25 It is difficult to know how much of this is due to increased frequency of screening, how much to increases

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Table 1. Invasive cervical cancer incidence rate by age group and year (England and Wales)

Age group (years)	Incidence rate (per 10^5 women/year) in					
	1972	1974	1976	1978	1980	1982
15–34	4·2	5·3	5·7	7·0	7·5	7·4
35–44	16·7	15·7	16·6	18·4	21·0	21·6
45–54	35·1	32·1	29·4	24·1	21·9	21·1
55–64	35·0	37·5	37·6	34·5	34·1	31·9
65+	26·1	28·0	26·7	26·6	26·7	26·1

in the proportion of women ever screened and how much to changes in the number of women in age groups eligible for screening. Clearly a woman must have been screened before a subsequent cancer can be recognized as having rapidly progressed through a pre-invasive phase.

We have attempted to estimate the increase in the number of cases of rapidly progressive cancer in young women that might be seen solely as a result of the above changes, if the proportion of such tumours had in fact stayed constant.

Method

We have adopted a stringent definition of 'rapidly progressive' as having a preclinical detectable phase (PCDP) of <12 months. The model with the simplest assumption – that the rate of transfer from pre-invasive to invasive disease is independent of the length of time that a tumour has been pre-invasive – gives rise to an exponential distribution for the PCDP. This also gives a satisfactory fit to data obtained from a cervical cancer screening programme (Parkin 1985).

For the sensitivity of cervical screening we have assumed a value of 0·80 which is comparable with the value of 0·76 given by Coppleson & Brown (1974) and the figure of 0·82 we estimate from the data given by Butler (1973).

It is then possible to derive a simple expression for the incidence rate, I_{12}, of invasive cervical cancer occurring within 12 months of a negative smear (see appendix):

$$I_{12} = I' \left\{\frac{(1-B)}{\lambda} [\exp(-12\lambda) - 1] + 12\right\}$$

I' = underlying incidence rate of potentially invasive disease;

$B = 1 -$sensitivity of the screening test (i.e. the false-positive rate);

$1/\lambda$ = mean duration of PCDP.

This only applies to women with a negative smear up to 12 months previously which for an annual rate is equivalent to the number of women screened per year. The underlying disease rate I' of early disease that will progress to invasion varies with age and its true values are unknown. We have estimated it for a given age band using the mean of the invasive disease incidence rate for that age band and the one above (Fig. 1). For this purpose national cancer registration data were used for the years 1972 and 1982 with corresponding population estimates (OPCS 1979, 1985).

The observed incidence rate of rapidly progressive cancers must be adjusted for the proportion of women who are screened. The proportion of women screened in each age group was estimated from the number of smears per year (Roberts 1982) and the population at risk in each age group (OPCS 1979, 1985) on the assumption that each woman had only one smear per year. This gives values of 14% and

Table 2. Number of women at risk ($\times 10^3$) at risk (England and Wales) by age and year

Age group (years)	Numbers of women ($\times 10^3$) at risk in					
	1972	1974	1976	1978	1980	1982
15–34	6543·8	6669·8	6844·5	7036·5	7198·7	7339·4
35–44	2818·9	2798·6	2752·5	2788·5	2896·2	3106·9
45–54	3081·2	3139·8	2979·5	2866·0	2783·9	2751·5
55–64	3004·0	2840·7	2906·0	2908·8	2864·7	2873·8
65+	4085·7	4205·4	4309·6	4411·1	4511·0	4576·3

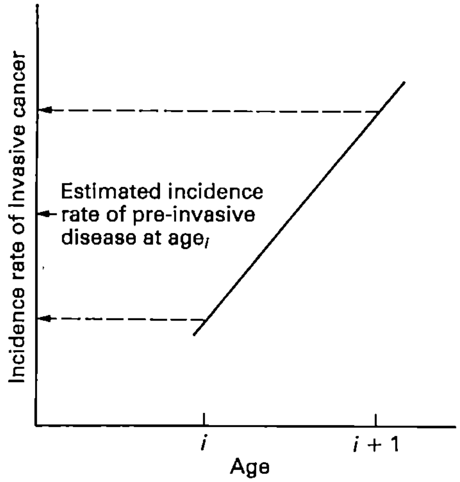
Incidence rate of invasive cancer
Estimated incidence rate of pre-invasive disease at age$_i$
i
$i + 1$
Age

Fig. 1. Estimation of pre-invasive disease incidence rate. Incidence rate of invasive disease in age band$_{i+1}$ reflects the incidence rate (and duration) of pre-invasive disease in earlier age bands. For simplicity we consider only adjacent age bands. It is reasonable to assume that invasive incidence age$_i$ < pre-invasive incidence age$_i$ < invasive incidence age$_{i+1}$. We then estimate pre-invasive incidence age$_i$ as ½[invasive incidence age$_i$ + invasive incidence age$_{i+1}$].

18% in 1972 and 1982 respectively for women <25 and a value of 25% for both years in women aged 25–34. The value of I_{12} adjusted for the proportion screened, Is_{12}, is given by:

$$Is_{12} = I_{12} \times \text{proportion screened.}$$

Results

Example calculation (assumed mean PCDP duration of 15 years):

λ = 0·0056/month

12λ = 1/15/year

On substituting into (iv) we find that

$I_{12} = I' \times 2{\cdot}713$.

(a) For women aged 15–24

In 1972: I' = 4·3/100 000/year
proportion screened = 0·14
Is_{12} = 1·64/100 000/year, or 56 cases in England and Wales.

In 1982: I' = 7·9/100 000/year
proportion screened = 0·18
Is_{12} = 3·85/100 000/year, or 143 cases in England and Wales.

(b) For women aged 25–34

In 1972: I' = 12/100 000/year
proportion screened = 0·25
Is_{12} = 8·27/100 000/year, or 260 cases in England and Wales.

In 1982: I' = 18/100 000/year
proportion screened = 0·25
Is_{12} = 12·32/100 000/year, or 422 cases in England and Wales.

Other results are displayed in Table 3.

The observed number of cases of invasive cervical carcinoma occurring within 12 months of a negative smear would have increased by 62% in women aged 25–34 and by 170% in women <25 between 1972 and 1982 purely as the result of overall increasing cancer incidence rates, changes in population structure and more extensive screening. This proportionate increase is independent of the PCDP chosen. In terms of a typical health district this would indicate that two or three cases would have occurred in 1982 with any of the assumed durations of the PCDP given in Table 3. Under any of these assumptions most would be cases that had been missed at screening. Using 1982 figures and assuming a PCDP lasting 10 years, a typical district should expect to have roughly one true rapidly progressive cervical cancer in a screened woman <35 years of age every 2 years or so. Four times as many apparent cases would turn out to be

Table 3. Effect of the preclinical detectable phase (PCDP) on the estimated incidence rate of rapidly progressive cervical cancers in women <35 years

Duration of PCDP (years)		Estimated incidence rate (per 10^5 women/year)				
Assumed mean	Median	Women <25		Women 25–34		False-negatives at screening (%)
		1972	1982	1972	1982	
25	17·3	1·56	3·68	7·89	11·76	90·85
15	10·3	1·64	3·85	8·27	12·32	85·58
10	6·93	1·73	4·07	8·73	13·01	79·73
5	3·46	2·00	4·71	10·10	15·06	65·93

failures of screening and, if available for review, the smear would prove to be positive or inadequate for a proper opinion. It should also be observed that if the exponential model is correct, the median duration of the PCDP will be considerably less than the mean, thus half the women would have a PCDP of <7 years if the average PCDP was 10 years.

Discussion

Much of the literature about rapidly progressive cervical cancer in young women is based on case reports or series of cases, often accrued over some years without information on the population at risk or the proportion with previous negative smears. Comparison of case reports is further hampered by a lack of data on background incidence rates and by variation in the interval regarded as 'recent'. For example, Paterson *et al.* (1984) reviewed 500 cases of invasive cervical carcinoma and found nine aged <35 who had had a negative smear <2 years previously, but they gave no data on the extent of screening at different ages.

Furthermore, many of the attempts to examine the natural history by fitting mathematical models to available data have suggested an inverse relation between age and duration of the in-situ phase. Coppleson & Brown (1975) found it necessary to allow the transition rate from in-situ to invasive disease to increase in older women, as did Habbema *et al.* (1980) in their more general model.

Given that natural variation exists in the duration of the PCDP, some cases are bound to occur with a short preclinical phase. A higher level of screening in younger women will mean that such cases are more likely to be detected; clearly studies which merely describe such patients cannot be used as evidence of a shorter PCDP in younger women. Berkeley *et al.* (1980) identified nine cases aged 20–39 with a 'normal' smear within 10 months of diagnosis, and Prendiville *et al.* (1980) reported just four similar cases, and yet it is suggested that 'the age of these patients lends support to the growing body of opinion in favour of a far more active cervical screening policy in young women'.

Bearing in mind the limitations of published data and adopting a strict definition of within 1 year for 'recent', our prediction seems broadly consistent with the single case in a woman <35 occurring over a 3-year period (Walker *et al.* 1983), the five cases over a 12-year period in women <35 (Paterson *et al.* 1984), four cases <35 in 1-year (Prendiville *et al.* 1980), and 10 cases in women <39 over 3 years (Berkeley *et al.* 1980). With a 2-year definition of 'recent' we may expect fairly similar figures: Berkowitz *et al.* (1979) had 15 such cases in women <35 over a 3·5-year period. Dunn & Schweitzer (1981) described 37 cases within 5 years of a negative smear in women <39 over a 5-year period but noted that overall 70% of the previous negative smears were within 2 years of diagnosis. Our estimate of the proportion of women screened may be a slight overestimate because it does not allow for repeat smears within 12 months. However, these are unlikely to follow a negative smear and the majority of smears are negative. Furthermore, correcting for this bias would slightly reduce the estimated number of rapidly progressive tumours that occur (both 'true' and 'apparent'), although the proportional change with time would be unaffected.

To confirm that a lesion has truly undergone rapid progression from a non-detectable to an invasive phase, the smear initially classed as negative should be reviewed to exclude the possibility of a false-negative. In reports where this has been done, the percentage of confirmed negative smears ('true' fast growers) has ranged from 12% (Dunn & Schweitzer 1981) through 44% (Berkeley *et al.* 1980) to 100% (Bamford *et al.* 1983). These studies, however, are all based on small numbers and may also be subject to selection bias because often slides have not been available for review and those that have been available may not be a representative sample. In addition we should like to stress the importance of blind reassessment of slides previously reported as negative. Little, if any, information is given on this point yet it must be obvious that even if the origin of the actual slide is coded, knowledge of the purpose of the study would make reclassification more likely, even if only to call a smear inadequate. Smears should be reassessed under working conditions as well as validated against a considered opinion.

Husain *et al.* (1974) have estimated that one-third of false-negatives are failures in assessing the smear, suggesting that imperfect methods of obtaining smears make a significant contribution to the occurrence of apparently fast-growing cancers. As far as errors in reading slides are concerned, Fowkes (1986) has commented on the problems of sustaining attention and the way in which expectancy affects performance, especially if a disease has low prevalence. With increased workload both factors are likely to decrease the sensitivity of screening and produce an even greater increase in apparent rapidly progressive cervical cancers. Not surprisingly there has been concern over the quality of service provision (Chamberlain 1984). If urging greater expenditure on screening, therefore, we should distinguish the mere possibility of a new form of cervical cancer from the undoubted existence of defective recall systems and a multiplicity of causes for a false-negative smear. Rylander (1977) has offered suggestions as to how the latter may be dealt with, while Paterson *et al.* (1984) note that a greater reduction in invasive cervical carcinoma would be produced by better quality control in the laboratory than by the introduction of annual smears.

Our results show that a considerable trend in numbers can be explained by secular trends in incidence, age structure and perhaps screening coverage. We do not deny the possibility of a new fast-growing kind of cervical cancer in young women but we do not feel that the evidence available is enough to support the idea. Our calculations suggest that the problem is one of service provision rather than perception of a new disease. They also provide a starting point for further studies of this very absorbing topic.

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Appendix

The incidence rate $I(t)$ of invasive disease at time t after a series of n negative smears is given by the following equation (Walter & Day 1983):

$$I_t = I' \sum_{i=0}^{n} B^{n-i} \int_{t-t_{i+1}}^{t-t_i} f(y).dy \tag{i}$$

where I' = underlying incidence rate of pre-invasive disease;
$B = 1 -$ sensitivity of the screening test, or the false-negative rate;
t_i = time of the ith screen ($t_o = -\infty$, $t_{n+1} = t$);
$f(y)$ = the probability density function of the duration of the preclinical detectable phase (PCDP) of the disease; possible choices might be a normal, log normal or exponential distribution.

The above expression can be simplified by ignoring the probability of two or more false-negative smears in succession (i.e. terms in B^2 and of higher powers) which is small compared with the probability of one false-negative screen. Equation (i) then becomes:

$$I_t = I' \; \{[\int_o^t f(y).dy] + [B\int_t^\infty f(y).dy]\} \tag{ii}$$

With an exponential distribution of PCDP durations, $f(y) = \lambda \exp(-\lambda y)$

The incidence rate I_{12} of invasive cervical cancer occurring within 12 months of a negative smear is given by integrating (ii):

$$I_{12} = \int_o^{12} I' \; \{\underbrace{[\int_o^t \lambda\exp(-\lambda y).dy]}_{\substack{\text{true rapidly}\\ \text{progressive cases}}} + \underbrace{[B\int_t^\infty \lambda\exp(-\lambda y).dy]}_{\substack{\text{cases not rapidly}\\ \text{progressive but}\\ \text{missed on screening}}}\}.dt \tag{iii}$$

which in turn simplifies to: $I' \; \{\frac{(1-B)}{\lambda}[\exp(-12\lambda) - 1] + 12\}$ (iv)

$$\%\text{ cases that are missed at screening} = \frac{B\,[1 - \exp(-12\lambda)] \times 100}{12\lambda - (1 - B)[1 - \exp(-12\lambda)]}$$

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A prospective study of colposcopy in women with mild dyskaryosis or koilocytosis

B. S. BOLGER, B. V. LEWIS

Summary. Colposcopy was performed in 91 women who had cervical cytology suggesting mild dyskaryosis or showing koilocytosis, all previous cytology having been normal. The final histological diagnosis was CIN III in 22%, CIN II in 18%, CIN I in 31%, koilocytosis alone in 14% and no abnormality in 15%. These results indicate that even with mild cytological atypia, a high proportion of patients will have more advanced disease when colposcopy-directed punch biopsy is performed.

Women presenting with cervical intraepithelial carcinoma (CIN) grade II or III (CIN II or III) require colposcopic assessment, and within most health authorities this is recognized and resources are made available. There is, however, growing evidence that women presenting with cytological changes suggesting mild dyskaryosis or changes associated with human papillomavirus (HPV) infection should also be colposcoped (Walker *et al.* 1986; Soutter *et al.* 1986; Singer *et al.* 1984; Davis *et al.* 1972; Nyirjesy 1972). These patients may not be referred to the colposcopy departments.

This study was designed to investigate the merits of early colposcopic assessment in women with mildly abnormal cytology.

Patients and methods

The study sample included all the women whose smears were processed in the Watford Cytology Department and in whom the smear report suggested mild dyskaryosis or showed koilocytosis, or both, and in whom all previous cervical smears had been normal. Before an abnormal cervical smear result is reported, the slide is reviewed by the chief cytology technician and consultant pathologist. There were three sources of cervical smears: general practice, family planning clinics and sexually transmitted disease clinics. The women were allocated to one of two management protocols according to whether the smear index number was even or odd. One group was asked to attend a research clinic for colposcopic examination and the other group entered the existing referral system in which repeat cytological examination was requested in 6 months time. On average there was an 8-week delay between the referral smear and the date of the first colposcopic examination.

Both groups of patients were followed by regular cytology, initially every 4 or 6 months and then annually if the cytology was reported as normal. This study reports the findings in the first group of women who were assessed colposcopically. The hospital ethics committee gave its consent to the study.

At the first visit, a gynaecological history was taken including enquiries about previous smear results. Where patients were not certain, their general practitioner was requested to provide this information. The purpose of the study was discussed with the women with emphasis on the need to understand the significance of mildly abnormal cytology.

At the first colposcopic examination, cervical cytology was repeated and endo- and ectocervical smears were taken by the doctor. A colpo-

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scopy was then performed using a Zeiss operating colposcope (OM5). Initial assessment was made using normal saline alone. Five per cent acetic acid was then applied and the findings described and recorded. Endocervical forceps were used if necessary. The characteristics of the squamo-columnar junction and the transformation zone, the colour change following application of acetic acid, the surface contour, vascular pattern and outline of any abnormal areas were observed. Lugol's iodine was then applied before the biopsy. The currently accepted criteria for colposcopic appearance of CIN and HPV infection were used (Villani *et al.* 1986). Directed punch biopsies were taken from any area with abnormal colposcopic characteristics. Women who were pregnant were excluded from the study group. All women were given a further appointment for 2–4 weeks later.

Any cervical abnormality was treated in this research clinic with the exception of the two following groups. One group comprised those women in whom colposcopic examination was incomplete, as judged by incomplete demonstration of the squamo-columnar junction. They had a cone biopsy if there was histological evidence of CIN II or worse; if CIN I or koilocytosis alone was noted, cervical cytology and colposcopy were repeated after 6 months. The second group included women whose biopsies showed CIN III, they had laser treatment at the regular colposcopy clinic by a senior colposcopist. This clinic also provided long-term colposcopic follow-up.

Women in whom a complete colposcopic examination was possible with histological confirmation of CIN II, CIN I or koilocytosis were treated by cryocautery. They were given a further appointment 4 months later.

The final category of women were those in whom colposcopy was entirely normal. If subsequent cytology was normal they were referred back to their general practitioner with a request to repeat a smear in 6 months.

Table 1. Age distribution in the study group

Age (years)	No. of women (n = 91)
<20	9
21–30	45
31–40	31
>40	6

Results

Over an 8-month period, 208 women were referred from the cytology department with mildly abnormal smears, and 109 were allocated to the colposcopy group. Of the 109 women 18 were excluded: five failed to attend (following two reminders), three did not want to attend, six were pregnant and four had had a previous abnormal cervical smear.

Thus the study sample consisted of 91 women, their average age was 28 years (Table 1). The referral smear showed koilocytosis alone in 29 (32%), mild dyskaryosis alone in 38 (42%) and mild dyskaryosis with koilocytosis in 24 women (26%).

Colposcopic examination was incomplete in eight women. Cytology and biopsies of the area of abnormal epithelium visible revealed CIN I in four and these women were kept under 6-monthly review. Biopsy showed CIN II in one and CIN III in three women. Histological examination of cone biopsies performed subsequently showed CIN III in all four. There was no invasion or koilocytosis. When the results in these eight women are added to the final histological diagnosis reached for the remaining 83 women, 20 (22%) were found to have CIN III, 16 (18%) had CIN II, 28 (31%) had CIN I, 13 (14%) had koilocytosis alone and 14 women (15%) were normal (Table 2). Subdividing the women according to the referral smear report makes little difference to the distribution of the final histological diagnosis.

Discussion

This study provides answers to the practical question of how to manage a woman with mildly abnormal cervical cytology. We found that a large percentage of women (39%) who had a single smear showing mild dyskaryosis or koilocytosis subsequently showed histological changes of CIN II or worse 8 weeks later. Such a histological diagnosis justifies removal or destruction of the abnormal epithelium in most women. Although our findings might represent rapid neoplastic change over the 8-week interval, it would seem more likely that the difference between the referral cytological diagnosis and the final histological diagnosis represents a failure in the technique of exfoliative cervical cytology. This is likely to be the result of incomplete sampling, as with adequate quality

Table 2. Final histological diagnosis by referral smear report in 91 women with mildly abnormal cervical cytology

	Final histological diagnosis				
Referral smear report	Normal	Koilocytosis	CIN I	CIN II	CIN III
Mild dyskaryosis (n = 38)	5 (13%)	5 (13%)	13 (34%)	7 (18%)	8 (21%)
Koilocytosis (n = 29)	5 (17%)	6 (21%)	7 (24%)	5 (17%)	6 (21%)
Mild dyskaryosis plus koilocytosis (n = 24)	4 (17%)	2 (8%)	8 (33%)	4 (17%)	6 (25%)
Total	14 (15%)	13 (14%)	28 (31%)	16 (18%)	20 (22%)

control in the laboratory, incorrect cytological diagnosis could not account for the difference noted. Alternatively, exfoliative cytology may have this defect independent of these possibilities.

In a retrospective study, Soutter *et al.* (1986) found that 49% of the women referred with a smear indicating mild dyskaryosis had a histological diagnosis of CIN II or worse. There was no difference in the final histological diagnosis between women who had a single smear showing mild dyskaryosis and those who had persistent cytological evidence of mild dyskaryosis.

There is, therefore, growing evidence that a clinically significant percentage of women with a cytological diagnosis suggesting mild dyskaryosis or wart virus infection have, in fact, more advanced underlying histological changes which justify early colposcopic assessment and appropriate treatment. Purely cytological follow-up should be discouraged because delayed treatment is likely to allow greater neoplastic change and possible invasion. However, long-term regular cytology follow-up is essential after treatment by cryosurgery or laser.

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Early pregnancy wastage after gamete manipulation

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Summary. The outcome of 1034 pregnancies in women who conceived after referral for infertility management in seven treatment groups is detailed. The mean early pregnancy wastage (before 20 weeks gestation) was 27% and ranged from 18% after AID (artificial insemination by donor semen) to 33% after IVF-ET (in-vitro fertilization and embryo transfer). These differences were not due to maternal age which was similar in all groups (means between 29·7 and 32·7 years). Excluding the AID group, there was a high rate of ectopic pregnancy which was significantly higher after GIFT (gamete intrafallopian transfer) and was only partly related to underlying tubal disease. Blighted ova was the main category of early pregnancy loss and was highest after AIH (artificial insemination by husband's semen). There was a higher rate of biochemical pregnancies after GIFT, PROST (pronuclear stage tubal transfer) and IVF-ET. Our findings confirm a high pregnancy wastage rate in subfertile women and highlight deficiencies in the sperm separation, gamete handling and IVF/embryo culture techniques.

In recent years, management of infertility has become increasingly sophisticated and many clinics worldwide are devoted entirely to the management of infertility problems. A range of techniques have been introduced which involve manipulation of the gametes in order to improve their chance of contact and subsequent fertilization. This has been combined with intensive monitoring of stimulated and unstimulated ovarian cycles to optimize the chance of inseminating the oocytes at the appropriate stage of maturation (Yovich *et al.* 1985*a*; Yovich 1988*a*). Some form of gamete handling or manipulation is involved with artificial insemination using frozen-thawed donor semen (AID) (Matthews 1984), artificial intrauterine insemination using a washed, precapacitated preparation of husband's semen (AIH) (Kerin *et al.* 1984), in-vitro fertilization and embryo transfer (uterine; IVF-ET) (Edwards & Steptoe 1983), gamete intrafallopian transfer (GIFT) (Asch *et al.* 1984), and pronuclear stage tubal transfer (PROST) (Yovich *et al.* 1987*a*).

With the development of IVF, closer monitoring of the luteal phase was established as part of most protocols (Yovich *et al.* 1985*a*; Hay *et al.* 1984) and enabled the earliest possible diagnosis of pregnancy, even before the due menstrual period. However, many IVF units learned to exhibit caution in diagnosing pregnancies based on hormonal evidence alone as up to 63% failed to continue as viable pregnancies (Hay *et al.* 1984; Seppala 1985; Romeu *et al.* 1987). These

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rates of loss are consistently greater than the rate of 10–20% for spontaneous conceptions diagnosed around 6–8 weeks gestation (Grudzinskas & Nysenbaum 1985).

At the PIVET Medical Centre, a comprehensive infertility service has been established and the present survey compares the pregnancy outcomes in a subfertile population between those who conceived after coitus (with or without ovarian stimulation) and those who conceived after regimens involving gamete manipulation.

Subjects and methods

The PIVET Infertility Management Service (PIMS) was established over a 5-year period, initially to perform IVF procedures after a successful pilot study undertaken during 1980/81 in the University of Western Australia (Yovich *et al.* 1982). The PIVET Medical Centre is a secondary and tertiary referral site for infertile couples and the PIMS programme applies diagnostic and treatment modes in a set protocol (Yovich 1988*a*). Treatments include microsurgical reconstructive tubal surgery (male and female), a range of ovarian stimulation methods (Yovich *et al.* 1987*b*), AIH, AID, IVF-ET, GIFT, PROST and other specific therapies such as endometriosis management. A total of 852 women was involved in the study.

Procedures involving gamete manipulation

AIH. This procedure involved 'washing' spermatozoa and replacing seminal plasma with a physiological culture medium such as Ham's F10 or Tyrode-based solutions similar to those applied for IVF. Hepes buffer was added earlier in the series to maintain pH levels around 7·4 but was discontinued because pregnancy rates did not differ when the pH drifted to between 7·6 and 7·8. The washed sperm concentrates (0·5 ml) were injected direct into the uterine cavity in the periovulatory period, using an inert catheter. Full details of the laboratory and clinical techniques have been described previously (Matson & Yovich 1988).

AID. Suitable samples of donor semen were diluted 1:1 in cryopreservatión medium which is a Tyrode-based solution containing 7·5% glycerol. The samples were frozen in 0·5 ml straws, applying standard routines in a programmable freezing machine using liquid nitrogen. The straws were stored at −196°C for periods ranging from 6 months to 4 years before use. Inseminations were performed intracervically at the beginning of the preovulatory, fertile mucus phase and continued until ovulation occurred (usually three or four daily inseminations).

IVF-ET. This procedure involved ovarian stimulation, usually with combinations of clomiphene citrate and human menopausal gonadotrophin (hMG) and oocyte recovery at laparoscopy, or more recently, using ultrasound-guided techniques, particularly the transvaginal ultrasound-guided method (Blackledge *et al.* 1986), 36 h after the estimated onset of an LH surge or a triggering injection of hCG. The full details of semen preparation, oocyte handling, dissection of the coronal coat, culture methods and embryo transfer techniques have been described previously (Yovich *et al.* 1984*a*).

GIFT. This technique involved laparoscopic oocyte recovery after ovarian stimulation similar to that used for IVF. Spermatozoa were also prepared in the same way except that the husband provided a sample 2 h before the scheduled oocyte recovery. After both gametes were suitably washed in culture medium, up to four oocytes were transferred into the fallopian tubes (preferably two per side) along with 100 000 spermatozoa in 0·5 ml of culture medium through a Teflon catheter at the same laparoscopy (Yovich *et al.* 1988*a*). For oligospermic men the semen preparation and insemination techniques were modified (Matson *et al.* 1987).

PROST. This modification of the GIFT technique was used where it was important to have evidence of fertilization as in couples with severe oligospermia, asthenospermia or antispermatozoal antibodies in either partner. Oocytes were recovered with the transvaginal ultrasound-guided method after ovarian stimulation. The gametes were treated exactly as for IVF-ET but once the pronuclear stage had been confirmed, up to three zygotes were transferred into the fallopian tubes at laparoscopy (Yovich *et al.* 1987*a*).

Pregnancy diagnosis

Pregnancy was diagnosed by an elevated serum β-hCG level on or after day 16 of the luteal phase with a significant rise at least 3 days thereafter (Yovich *et al.* 1986*a*). The assay for β-hCG had a minimum detection level of 2·5 i.u./l and all levels of $\geqslant$ 25 i.u./l standardized against the 2nd International standard (61/6) were considered to diagnose a pregnancy. Serum oestradiol (E_2) and progesterone were measured in the same sample and were also required to be in the appropriate pregnancy range (>650 pmol/l and >37 nmol/l respectively) (Yovich *et al.* 1986*a*). All the patients included in this study had a pregnancy diagnosed within 7 days of the missed menstrual period and had β-hCG, E_2 and progesterone measured weekly throughout the first trimester (Yovich *et al.* 1985*a*, 1986*a*). Ultrasound assessment was performed at 7 weeks gestation. Pregnancies with falling hormone levels and no detectable gestational sac on ultrasound were considered biochemical pregnancies and were not curettaged. Pregnancies which reached the stage of a gestational sac at 7 weeks without a viable intrauterine fetus were regarded as blighted ovum pregnancies. Pregnancies that miscarried after ultrasonic detection of fetal heart movement were diagnosed as miscarriages. After 20 weeks gestation fetal or neonatal death was categorized as a late pregnancy loss. Ectopic gestations can be diagnosed very early by elevated β-hCG, progesterone and E_2 levels without a definite intrauterine gestational sac detectable on ultrasound at 7 weeks. Where ectopic pregnancies were diagnosed, the medical histories of the patients were checked for evidence of tubal disease. Previous tubal reconstructive surgery, fibrous periadnexal adhesions and isthmic nodules were categorized as 'known' disease; tortuous fallopian tubes, sparse filmy pelvic adhesions and impeded flow of dye on pertubation of apparently normal tubes at laparoscopy were diagnosed as 'suspected' tubal disorders.

Table 1. Pregnancy outcome in subfertile patients who conceived without gamete manipulation

Pregnancy outcome	Unstimulated cycles (n=462) *n*	(%)	Stimulated cycles* (n=175) *n*	(%)
Biochemical	9	(2·0)	5	(2·9)
Blighted ovum	56	(12·1)	27	(15·4)
Miscarriage	23	(5·0)	12	(6·9)
Ectopic	23	(5·0)	9	(5·1)
Termination	3	(0·7)	0	(—)
Delivered at $\geqslant$20 weeks	348	(75·3)	122	(69·7)
Age of women (years) mean (range)	30·9	(19–44)	30·5	(23–39)

*Ovarian stimulation with clomiphene citrate and/or hMG

Luteal support therapy

Some women in each of the categories conceived whilst receiving luteal support therapy with hCG, progesterone or medroxyprogesterone acetate. These studies have been reported previously (Yovich *et al.* 1984*b*, 1985*b*; Yovich 1988*b*). Approximately one third of the patients in each of the categories received luteal support therapy and where hCG had been given, pregnancy diagnosis required a serial rise in β-hCG estimations during the week after treatment stopped.

Data analysis

The mean ages at the time of pregnancy diagnosis were compared between the groups using Student's *t*-test. Statistical analysis of pregnancy outcomes used the χ^2-test in appropriate contingency tables with Yates' correction where applicable.

Results

The data presented form part of a prospective analysis and include all patients managed in the PIMS programme over the 5-year period whose pregnancies were diagnosed within 7 days of the due menstrual period before October 1986, so that all pregnancy outcomes were known and could be categorized into early losses (before 20 weeks) and subsequent deliveries (beyond 20 weeks). A total of 1034 pregnancies were diagnosed of which 755 (73%) continued beyond 20 weeks gestation. The overall early pregnancy wastage was 27% and ranged from 18% for AID patients to 33% for those conceiving after IVF. Table 1 shows the pregnancy outcomes for the subfertile patients who conceived without gamete manipulation with or without ovarian stimulation. Of the 462 pregnancies conceived without ovarian stimulation, the diagnoses for infertility were unexplained in 304, non-occlusive tubal disorders in 83 (37 of whom had previous tubal reconstructive surgery), oligospermia in 27, female antispermatozoal antibodies in 5, male antispermatozoal antibodies in 5, pelvic endometriosis in 24 and negative postcoital tests in 14. Of the 175 women who conceived after ovarian stimulation, the diagnoses for infertility were unexplained in 120, non-occlusive tubal disorders in 26 (9 of whom had previous reconstructive tubal surgery), oligospermia in 8, male antispermatozoal antibodies in 2, pelvic endometriosis in 12, and negative postcoital tests in 7. There were no significant differences between the two groups and they were statistically homogeneous in respect of early pregnancy outcomes. Known underlying tubal disorders were present in 11 of the 23 ectopic pregnancies in women having no ovarian stimulation and in 3 of the 7 who did receive ovarian stimulation. Of the 348 pregnancies that continued beyond 20 weeks in the unstimulated group, 7 (2%) were multiple (all twins). The frequency of multiple pregnancy in the stimulated group was significantly higher with 17 twins and 6 triplets (23/122; 18·9%). A few multiple pregnancies miscarried before 20 weeks and they are included in the miscarriage data.

The pregnancy outcome for those patients receiving gamete manipulation in the various treatment regimens is summarized in Table 2. Overall, the pregnancy wastage for the groups having gamete manipulation was variable and a function of the techniques applied (excluding the few data available for PROST, χ^2, = 8·31; $P<0{\cdot}05$). There were no differences within the groups regarding miscarriages or other forms of pregnancy wastage in relation to luteal support therapy. The AID pregnancies continued beyond 20 weeks more often than AIH, IVF-ET, GIFT and PROST pregnancies ($P<0{\cdot}05$) but there were no differences between these latter groups, as defined in the present study. In earlier days we had diagnosed pregnancies at β-hCG levels of 10 i.u./l which would have identified a further 54 biochemical pregnancies in the IVF-ET series. It is no longer considered relevant as only 3 of 1034 pregnancies included in this study and which proceeded to the clinical detection stage (one ectopic and two blighted ova) had an initial β-hCG level <25 i.u./l on days 16–19 which subsequently elevated into the diagnostic range.

Overall, biochemical pregnancies were diagnosed significantly more often after gamete manipulation than after coitus ($P<0{\cdot}005$) and the IVF-related procedures had a significantly higher rate ($P<0{\cdot}005$) than the combined artificial insemination procedures (AID and AIH).

Blighted ovum pregnancies constituted the main type of pregnancy wastage in all groups, with rates ranging from 8% in the GIFT group to 20% in the AIH group but the differences did not reach statistical significance ($P>0{\cdot}05$). Miscarriage of a viable fetus was a relatively uncommon event in all the groups although it occurred significantly more often in the GIFT group than in the IVF-ET group ($P<0{\cdot}025$). This was attributed to the second trimester miscarriages of multiple pregnancies in the GIFT series. Of the 51 GIFT pregnancies that continued beyond 20 weeks, 15 (29%) were multiple, which was higher than the corresponding proportion of 16% (13 of 81) in the IVF-ET series (χ^2 3·34; $P<0{\cdot}05$).

Excepting the AID group, the rates of ectopic pregnancies were high in all groups (5–9%) but the only significant difference was between the AID and GIFT groups ($P<0{\cdot}025$). The association between underlying tubal disease and ectopic pregnancy is shown in Table 3. This ranged from nil in the AIH series to 67% in the IVF-ET group and 83% in the unstimulated spontaneous conception group when the known and suspected tubal factors are combined.

Discussion

This study has focused on the pregnancy outcome of subfertile patients who subsequently conceived following a range of treatments involving gamete manipulation, as well as those who conceived after coitus, with or without ovarian stimulation therapy. Pregnancy outcome between groups was not due to differences in maternal age. Ovarian stimulation did not affect the outcomes in pregnancies conceived after coitus although a significantly higher proportion of multiple pregnancies was conceived after stimulation (19% compared with 2%). It was of particular interest to note that ectopic pregnancies occurred in 5% of conceptions after coitus but in fewer than 1% of the AID pregnancies. It is relevant that female infertility factors were uncommon in the AID patients whereas all other groups, including the AIH series, contained a high proportion with known female infertility factors (Yovich 1988*a*). The rate of early pregnancy wastage was 18% in the AID series and 25–30% in the coital-conception group. These rates are higher than expected in a general population (Grudzinskas & Nysenbaum 1985) but are consistent with the careful analysis of early pregnancy wastage detailed by Jansen (1982) in women conceiving after infertility treatment which included ovulation induction, endometriosis therapy and tubal or uterine surgery. Given the low proportion of biochemical pregnancies detected in the coital-conception and AID series, we would agree with Jansen's conclusion that the increased risk of early pregnancy wastage is not explicable merely by heightened surveillance for early pregnancy losses.

The most striking findings in this study were the subcategories of early pregnancy wastage in patients conceiving after gamete manipulation. The rates of pregnancy wastage were similar in the gamete manipulation groups except for the AID group which had significantly fewer losses. Blighted ovum pregnancies were the main subcategory and were noted to be most frequent in the AIH group. Since the recent advent of high resolution real-time ultrasound scanning, it is now more widely appreciated that most of the early pregnancy losses (≥85%) are of the type defined here as 'blighted ova' (Wilson *et al.* 1984; Yovich *et al.* 1986*b*). Although there is a widespread view that blighted ova pregnancies are largely the consequence of chromosomal aberrations (Boue *et al.* 1975; Poland *et al.* 1977; Simpson 1980), these conclusions were based on studies undertaken on aborted material, generally late in the first trimester. Our study differs in that many of the cases exhibited degeneration *in utero* without the usual clinical manifestations of miscarriage. Nonetheless, the finding raises concern about the sperm separation techniques used for AIH. The techniques used allow some spermatozoa of low motility to reach the tube and fertilize an ovum; but we do not know enough about the relevance of spermatozoal motility and its relation to chromosomal normality.

In the IVF-ET, GIFT and PROST series, much of the early pregnancy wastage was caused by biochemical pregnancies. In reporting IVF pregnancy data, centres vary in their inclusion of such cases (Jones *et al.* 1983). Many await ultrasound confirmation of a gestational sac, others

Table 2. Pregnancy outcome in subfertile patients conceiving after gamete manipulation

	Technique applied									
	AID[c] (n=116)		AIH (n=76)		IVF-ET (n=119)		GIFT (n=76)		PROST (n=10)	
Pregnancy outcome	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Biochemical[a]	2	(1·7)	3	(4·0)	11	(9·2)	7	(9·2)	2	(20·0)
Blighted ovum	13	(11·2)	15[c]	(19·7)	17	(14·3)	6	(7·9)	—	
Miscarriage	5	(4·3)	3	(4·0)	1	(0·8)	5	(6·6)	1	(10·0)
Ectopic[b]	1[c]	(0·9)	4	(5·3)	9	(7·6)	7	(9·2)	—	
Delivered	95[c]	(81·9)	51	(67·1)	81	(68·1)	51	(67·1)	7	(70·0)
Age of women (years) mean (range)	31·8(22–41)		31·8(24–43)		32·5(22–44)		32·7(21–40)		29·7(21–37)	

[a] IVF-ET, GIFT, PROST>AH, AID ($P<0{\cdot}005$).
[b] IVF-ET, GIFT>AIH, AID ($P<0{\cdot}025$).
[c] AID: fewer ectopics and more deliveries ($P<0{\cdot}05$)

Table 3. Tubal anomalies in subfertile patients developing ectopic pregnancies

	Coitus						
Tubal condition	Spontaneous cycle	Stimulated cycles	AID	AIH	IVF-ET	GIFT	PROST
Normal	4	3	—	4	3	3	—
Suspect	6	4	1	—	2	1	—
Known disorder	13	2	—	—	4	3	—
Total	23	9	1	4	9	7	—

rely on a higher threshold of β-hCG concentrations and/or the maintenance of these levels for variable periods before including the case in the pregnancy data analysis. Two other IVF units which have been reporting for more than 5 years show that early pregnancy wastage occurs in up to 63% of pregnancies diagnosed after IVF-ET and that a large proportion of these are biochemical (Hay *et al.* 1984; Romeu *et al.* 1987). Initially, we attributed the high proportion of biochemical pregnancies to the intensity of monitoring of the luteal phase proceeding into the first trimester of pregnancy. However, the diagnostic method, including the degree of monitoring, was the same for all the groups within this study. We had noted a higher proportion of biochemical pregnancies, mainly in the IVF-ET series, when the first hCG level was in the range of 10–24 i.u./l. This is no longer considered relevant as only 3 of 54 such pregnancies reached the stage of clinical detection and none continued beyond the first trimester. In the present study, the accurate diagnosis of pregnancy includes three components: a serum β-hCG level of ≥25 i.u./l based on the second International Reference Standard (61/6) on or after day 16 of the luteal phase; a further significant rise of hCG at least 3 days later and an associated increase in progesterone and oestradiol concentrations. However, even with these criteria, it is clear that biochemical pregnancies are a feature of IVF-ET and related procedures, using the techniques currently applied.

This experience with IVF-ET in man is matched by reported studies in animals. One such study in rats (Vanderhyden *et al.* 1986) showed a 35% greater loss of IVF embryos at the time of implantation above that where embryos were fertilized *in vivo* and then flushed for transfer into recipient rats. Overall, such animal studies mirror the experience in man by showing that <25% of embryos fertilized *in vitro* will lead to term fetuses. With GIFT we have achieved pregnancy rates of 32% and 36% in a large series of women and this is significantly higher than in our IVF-ET series run concurrently (Turner & Yovich 1987; Yovich *et al.* 1988*b*).

The PROST technique resembles IVF-ET and GIFT in that both gametes are manipulated *in vitro* through the fertilization and early culture stage before the pronuclear oocytes are transferred to the fallopian tubes. Although our series is small, the early results indicate that PROST has a lower biochemical pregnancy rate than IVF-ET. We now have the outcomes for 76 PROST pregnancies; 57 continued beyond 20 weeks, six were biochemical, seven were blighted ova, there were three miscarriages and three ectopic pregnancies. The data resemble the GIFT series (but fewer ectopics) and suggest that the main problem with IVF-ET in man relates either to the extended in-vitro culture of embryos or, alternatively, that the intrauterine environment is unfavourable for embryos 2 days after oocyte recovery. Yovich & Matson (1987) found a considerable fluctuation in the rate of biochemical pregnancies, with a trend showing an inverse relation between the biochemical pregnancy rate and the overall IVF-ET pregnancy rate. It remains likely that the problem of biochemical pregnancies reflects intrinsic deficiencies or adverse factors within the fertilization and culture system, causing poorer embryo quality with increasing exposure to the in-vitro environment.

The significantly higher rate of miscarriage in the GIFT series was attributed to the increased number of multiple pregnancies which miscarried before 20 weeks, including twin, triplet and one septuplet pregnancy (Yovich *et al.* 1988*a*).

The subject of ectopic pregnancies deserves special mention. The rate was high in all the subfertile groups except in the AID group. Yovich *et al.* (1985*c*) indicated that one of the causes of ectopic pregnancy was related to the embryo transfer technique. Our GIFT series had a significantly higher rate of ectopic pregnancy than the IVF-ET series and as over half were associated with tubal disease, this could be the cause. Nonetheless, we have shown (Turner & Yovich 1987) that patients who fail to conceive after microsurgical repair of the fallopian tubes can be considered for GIFT but should be counselled about their increased chance of ectopic pregnancy. Tubal disorders seem to be associated with most ectopic pregnancies but it is possible that ovarian stimulation may affect tubal motility (Yovich *et al.* 1984*c*) and this could explain our finding that most of the ectopic pregnancies in the stimulated coitus group were not associated with known tubal disorders. Alternatively, there may be unrecognized tubal defects such as isthmic diverticulae and partial cornual or isthmic obstructions due to nodules. Advances in diagnostic techniques will be required to explain fully the high rate of ectopic pregnancy in subfertile populations.

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Screening of the fetal heart rate in early labour

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Summary. The fetal heart rates (FHR) of 588 women admitted in labour, or in early labour after induction, were screened for up to 60 min (average 17 min) using computerized numerical analysis. Decelerative records with normal FHR variation occurred in eight (1·4%) and were not associated with acidaemia or depression at birth. Forty women (6·8%) had fetuses with a reduced FHR variation. They were more likely to be in spontaneous labour, to be at an earlier gestational age, and to be delivered operatively for fetal distress of babies that were more hypoxaemic and acidaemic. However, none of the fetuses with the least variable FHR patterns required resuscitation at birth or special care. The results of the screening procedure were not fully concealed and could have influenced clinical management. This is unlikely to have caused the associations that were observed, but could have reduced an association between low FHR variation and poor outcome. We conclude that there is a basis for a randomized controlled trial of FHR screening in early labour, but that this would need to be large (more than 10 000 patients) to test the benefit of detecting the most sinister pattern (decelerations with reduced variation) of which only one example was found in this study.

Continuous monitoring of the fetal heart rate (FHR) in labour is a standard practice to detect fetal distress. The accuracy is not high; for this reason an abnormal trace is not a good guide to intervention (Banta & Thacker 1979) but can be used to select cases for further investigation by measuring the pH of a fetal scalp blood sample. Adverse features include excessively fast or slow rates, reduced or absent variability, and late or variable decelerations (Beard *et al.* 1971). Decelerations are the easiest feature to discern and, perhaps for this reason, have been considered to be the most significant clinical abnormality. They are found in about 6% of monitored labours (Krebs *et al.* 1980) and may often result from reversible side-effects of oxytocin administration or epidural analgesia (Schifrin 1972). However, changes in FHR variability may be a more important feature for clinical diagnosis (Court & Parer 1984), although harder to recognize and quantify.

It is not clear which patients are most likely to benefit from continuous FHR monitoring in labour. Its use may increase the rates of operative intervention (Haverkamp *et al.* 1979) and possibly predispose to infection of the genital tract. Randomized controlled trials in high-risk patients (Renou *et al.* 1976; Haverkamp *et al.* 1979) or low-risk patients (Kelso *et al.* 1978; MacDonald *et al.* 1985) show no benefit of monitoring in terms of perinatal survival but do show higher umbilical artery blood pH and Po_2 values (Renou *et al.* 1976) and fewer seizures

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(MacDonald *et al.* 1985) or other neurological abnormalities (Renou *et al.* 1976) in the neonatal period. Continuous monitoring detects more acidaemic fetuses determined by fetal blood sampling, but has no effect on the frequency of low Apgar scores (MacDonald *et al.* 1985). But Apgar scores are a very general description of the state of the child at birth and correlate poorly with umbilical blood acid-base status (Sykes *et al.* 1982).

The present evidence does not justify the routine monitoring of all women in labour. The question arises whether a brief recording of the FHR can be used to select those women at the beginning of labour who need continuous monitoring. Although FHR patterns can be assessed by eye it is more precise to use numerical analysis. We have previously devised a technique for screening the FHR in antenatal patients with an on-line computerized system (Lawson *et al.* 1984). The record length was shortest for the most variable traces and the sampling time extended so that the least variable patterns were identified over the longest time. We have now applied this screening technique to women on admission in labour. The study was designed to determine whether a short, numerically analysed, FHR trace, on admission to labour ward, identifies fetuses who might benefit from continuous monitoring throughout labour.

Subjects and methods

The study was begun and completed in 1 year. This limited the sample size to 600 women. All women with singleton pregnancies of more than 36 weeks gestation who were admitted in labour to the John Radcliffe Hospital, Oxford, were eligible for inclusion. The criteria for diagnosing spontaneous labour were deliberately imprecise so that the use of the screening procedure could reflect routine clinical practice in a busy unit. Women admitted with spontaneous painful contractions were included without further restriction and the diagnosis of labour was made retrospectively. Women who were not delivered within 24 h of the screening record were deemed not to have been in labour and were excluded from the final analysis.

Women admitted for induction presented a different problem. Because their labours usually began in daylight hours they were more accessible for study, and the sample was biased towards their inclusion. For this reason selection of women with induced labours was stopped once more than 200 had been screened, out of the planned total of 600 women. Labour was more strictly defined to determine the timing of the screening after induction. Women who did not have a prostaglandin pessary were not screened until at least 1 h after the artificial rupture of membranes and only when regular painful contractions were recorded at least every 5 min. If a prostaglandin pessary was used, screening was deferred until there were painful regular contractions at least every 5 min, with either ruptured membranes or a cervix, fully effaced in women who had not laboured before, or 3 cm dilated in those who had laboured.

Selection was determined by the availability of both the screening monitor and the principal investigator or one of a small group of midwives trained in the screening procedures. It was not possible for the record to be taken 'blind' because in some cases it was clinically indicated and in all its duration was obvious. In patients where FHR monitoring had already begun the study record was substituted for the appropriate time and made available for clinical management. Otherwise only if the screening record showed abnormalities that would normally require intervention (immediate delivery or a fetal blood sample) were the medical staff shown the screening trace.

The FHR recording was taken using an external ultrasound transducer unless an internal fetal scalp electrode was already in use. The correlation between the two methods is high (Lawson *et al.* 1983).

The FHR was recorded using a Hewlett–Packard 8040A Doppler ultrasound monitor with a wide range transducer (1527A). The pulse intervals and the measurements from the external uterine tocodynamometer were analysed using a Nascom 2 microprocessor, as described previously (Lawson *et al.* 1984). The first analysis was made after 10 min, and it was repeated at intervals of 5 min or, after the first 30 min, of 10 min. During each analysis a baseline was fitted (Dawes *et al.* 1982*a*) and the FHR variation measured as the mean range of the pulse intervals calculated about the baseline, minute by minute (Dawes *et al.* 1985). Decelerations (>10 beats/min for 1 min, or >20 beats/min for 30 s) were identified and excluded from the computation of FHR variation. The area of decelerations below the baseline was measured in terms of the number of lost beats.

The length of the record was adjusted so that those with the least variable FHR had the longest screening time. The criteria for stopping were based on the results of the first 50 patients (25 with spontaneous labour and 25 with induced labour). These records lasted for 60 min and were analysed to give the estimated centiles of FHR variation. Thereafter the screening procedure was stopped at 10, 15, 20 or 30 min if, at these times, the minute range reached or exceeded 44, 33, 27 or 27 ms respectively, corresponding to the 50th, 25th, 10th and 5th centiles at these times. Each centile applies to a different frequency distribution (for different record durations), which is why the limits for the 10th and 5th centiles can be numerically identical. Thus 5% of patients, with the least variable traces, were to be screened for 60 min. However, if during the screening time a large deceleration (⩾20 lost beats) was identified then screening continued for 60 min regardless of the FHR variation.

The screening was carried out after a verbal explanation of the purpose of the study and the woman's agreement to participate. The study was approved by the Central Oxford research ethics committee.

Statistical analyses were by the χ^2-test with Yates' continuity correction, Fisher's exact test, and Student's *t*-test for the differences between two means. Trends in proportions were tested by the χ^2-test (Armitage 1971). Two-tailed tests were used except where stated.

Results

A total of 601 women was screened but 13 were excluded because they failed to give birth within the next 24 h (i.e. were 'not in labour'). Our data describe the remaining 588 women, of whom 305 (51·9%) were primigravid. Of the 588 women 203 (34·5%) were induced at a mean gestational age of 281 (SD10) days and the remaining 385 women laboured spontaneously at a mean gestation of 278 (SD8) days. Indications for induction were mainly 'prolonged pregnancy' (66 patients) or 'social reasons' (53 patients). The average duration of screening was 17·3 min.

Forty-eight women met the criteria for the longest 60-min screening record, 40 on the basis of lack of FHR variability and eight because of the presence of one or more large decelerations associated with normal variation.

Retrospectively, these decelerative traces were further classified by visual inspection. Only one showed late decelerations; in another the pattern suggested a recording artefact; in the remaining six the decelerations were variable in form and temporal relation to uterine contractions. The group differed from the rest only in a shorter mean interval from screening to delivery, 4·7 (SD4·2) h (Table 1), implying that labour was more advanced at the time of recording. Induction or augmentation was not used more frequently. The gestational age, birthweight, neonatal condition and cord blood gas measurements were all similar to those of the rest of the group. For these reasons it was decided that the detection of decelerations without loss of variability was not clinically useful. The group was therefore retrospectively incorporated, on the basis of their measurements of mean range, into the other screening categories—one each of 10, 20 and 30 min, and 5 of 15 min duration.

The remaining 40 women with the flattest (and longest) traces were at a significantly earlier gestational age (t=2·28, P <0·05). In theory this association might be explained because women with fetal problems, associated with unreactive FHR patterns, were induced before term. In practice this was not the case. Significantly more (33 or 82·5%) laboured spontaneously (χ^2=4·72, P<0·05), although the mean difference was small (3–5 days). The differences in the FHR pattern could not be attributed to the stage of cervical dilatation at the time of screening, whether or not the membranes had ruptured, or the use of oxytocin (Table 1). There were no significant differences between the groups screened for ⩽30 min.

The details of delivery and perinatal outcomes are shown in Table 2. The proportion of patients delivered operatively for fetal distress increased with the duration of screening (χ^2=7·11, P<0·01). The birthweights and weights for gestational age were similar in all the groups, as were the Apgar scores at 1 and 5 min, and the frequency of intubation for resuscitation at delivery. None of the 40 infants screened for 60 min was admitted to the special care nursery. One baby, screened for 30 min in early labour, died of congenital malformations associated with Trisomy 18. There were no other perinatal deaths.

Cord blood gas analyses were obtained in about three-quarters of the patients. The distribution of the missing values was not entirely

Table 1. Characteristics of women and labours according to duration of record

	Duration of screening (min)				
Variable	10 (n=273)	15 (n=163)	20 (n=79)	30 (n=33)	60 (n=40)
Gestational age (days)	279 (8·6)	279 (8·6)	279 (8·7)	281 (7·5)	276 (8·0)
Nulliparae	138 (50·5%)	81 (49.7%)	45 (57·0%)	19 (57·6%)	22 (55·0%)
Spontaneous labour	160 (58·6%)	118 (72·4%)	54 (68·4%)	20 (60·6%)	33 (82·5%)
Rupture of membranes before screening	174 (63·7%)	104 (63·8%)	55 (69·6%)	21 (63·6%)	26 (65·0%)
Oxytocin before screening	43 (15·8%)	27 (16·6%)	18 (22·8%)	4 (12·1%)	3 (7·5%)
Interval to delivery (h)	6·6 (4·8)	5·8 (4·1)	6·5 (4·0)	6·7 (4·3)	7·2 (4·9)

Results are means (SD) or n (%) as appropriate.

Table 2. Monitoring, delivery and neonatal outcome by duration of record

	Duration of screening (min)				
Variable	10 (n=273)	15 (n=163)	20 (n=79)	30 (n=33)	60 (n=40)
Continuous FHR monitoring after screening	80 (29·3%)	41 (25·2%)	27 (34·2%)	8 (24·2%)	17 (42·5%)
Mode of delivery					
Spontaneous	214 (78·4%)	126 (77·3%)	64 (81·0%)	22 (66·7%)	27 (67·5%)
Forceps	49 (17·9%)	26 (16·0%)	12 (15·2%)	8 (24·2%)	10 (25·0%)
LSCS	10 (3·7%)	11 (6·7%)	3 (3·8%)	3 (9·1%)	3 (7·5%)
Delivery for fetal distress	7 (2·6%)	5 (3·1%)	6 (7·6%)	2 (6·1%)	4 (10·0%)
Birthweight (g)*	3390 (421)	3372 (433)	3444 (507)	3346 (454)	3332 (508)
Centile <10th	21 (7·7%)	15 (9·2%)	6 (7·6%)	5 (15·2%)	6 (15·0%)
Low Apgar score					
≤6 at 1 min	31 (11·4%)	23 (14·1%)	13 (16·5%)	4 (12·1%)	9 (22·5%)
≤6 at 5 min	1 (0·4%)	0 (0·0%)	1 (1·3%)	1 (3·0%)	0 (0·0%)
Intubation at birth	16 (5·9%)	5 (3·1%)	1 (1·3%)	1 (3·0%)	0 (0·0%)
Admitted to special care nursery	8 (2·9%)	5 (3·1%)	1 (1·3%)	1 (3·0%)	0 (0·0%)
Perinatal deaths	0 (0·0%)	0 (0·0%)	0 (0·0%)	1 (3·0%)	0 (0·0%)

*Mean (SD).
LSCS, Lower segment caesarean section.

uniform, but there were no significant differences in gestational age, mode of delivery, reason for delivery, Apgar scores or intubation rates between those who did, or did not, have cord blood gases measured. There were no significant differences in umbilical vein blood gas values. There was a trend for the artery blood pH to diminish with the increasing duration of early labour screening (Table 3). The trend in terms of the proportion of patients with an umbilical artery blood pH of <7·20 was statistically significant (χ^2=7·19, P<0·01). When the group screened for 60 min was compared with the rest the differences were significant (pH:

t=2·31, P <0·05; $P\mathrm{o}_2$: t=2·50, P <0·02). Three (10%) of this group had an artery base excess of >20 mmol, compared with one (0·2%) of the remainder (P <0·01, Fisher's exact test with prior hypothesis). Thus the group screened for 60 min because of unreactive traces was significantly more hypoxaemic and acidaemic at delivery than the others, although this appeared to have had no clinical consequences.

The 40 patients in the 60-min category were subdivided by analysing the FHR variation in the first and second 30 min separately, to distinguish those in whom the FHR variation remained consistently low from those who showed a transition to an active phase of the fetal sleep cycle in the second 30 min. In 14 patients the mean minute range remained <27 ms (unreactive for 60 min: Table 4) whereas in 18 it rose to more than 27 ms, and in 13 to more than 32 ms (unreactive for 30 min only: Table 4). In eight patients the intention to screen for 60 min was not achieved because of clinical indications in three, maternal request in two, and computer failure in three, and the variability of the second half of the 60-min record was not available. The outcome of labour and delivery was analysed by

Table 3. Screening category by umbilical artery blood gas measurements

	Duration of screening (min)				
	10 (n=273)	15 (n=163)	20 (n=79)	30 (n=33)	60 (n=40)
Per cent with no reading	23·8%	27·6%	21·5%	51·2%	25·0%
pH	7·22 (0·09)	7·22 (0·09)	7·19 (0·08)	7 17 (0·11)	7·15 (0·14)*
$P\mathrm{co}_2$ (mmHg)	47·9 (10·5)	46·9 (9·7)	50·1 (10·7)	54·7 (11·0)	54·1 (13·0)
$P\mathrm{o}_2$ (mmHg)	13·6 (6·6)	13·4 (6·5)	12·0 (5·8)	13·5 (7·5)	10·9 (5·2)*
Base deficit >10 mmol/dl	42 (20.2%)	22 (18·6%)	20 (32.3%)	5 (31·3%)	10 (33·3%)

*Significant decreases in pH (P<0·05) and $P\mathrm{o}_2$ (P<0·02)
Results are means (SD) or n(%) as appropriate.

Table 4. Outcome by category of fetal heart rate screening

Outcome	Short traces (n=548)	Unreactive for 60 min (n=14)	Unreactive for first 30 min only (n=18)	Inadequate information after 30 min (n=8)
Mode of delivery				
Spontaneous	426 (77·7%)	11 (78·6%)	11 (61·1%)	4 (50·0%)
Forceps	95 (17·3%)	2 (14·2%)	7 (38·9%)	2 (25·0%)
Caesarean section	27 (4·9%)	1 (7·1%)	0 (0·0%)	2 (25·0%)
Operative delivery for fetal distress	20 (3·6%)	1 (7·1%)	2 (11·1%)	1 (12 5%)
Birthweight (g)	3390 (439)	3181 (428)	3369 (515)	3515 (607)
Gestational age (days)	279 (8·5)	274 (7·8)	276 (8·0)	280 (8·3)
Birthweight <10th centile	47 (8·6%)	3 (21·4%)	3 (16 7%)	0 (0·0%)
Umbilical artery blood				
No measurement	144 (26·3%)	4 (28·6%)	4 (22·2%)	2 (25·0%)
pH	7·21 (0·09)	7·08 (0·20)*	7·17 (0·10)	7·23 (0·04)
$P\mathrm{co}_2$ (mmHg)	48·2 (10·5)	55·6 (12·9)	56·0 (14·0)	47·3 (9·9)
$P\mathrm{o}_2$ (mmHg)	13·3 (6·5)	11·8 (5·8)	10·0 (4·4)	11·1 (6 4)
Base deficit >10 mmol/dl	89 (22·0%)	7 (70·0%)	2 (14·2%)	1 (15·7%)

*t=2.43, P<0·01 (with prior hypothesis) compared with group with short traces.
Results are means (SD) or n (%) as appropriate.

these categories to determine whether the group with persistent lack of variability showed more adverse features (Table 4). This was found with respect to a significantly lower average birthweight and gestational age compared with those with short FHR records (t=1·80, P<0·05; t=2·36, P<0·01 respectively; using one-tailed tests). This group also had the lowest umbilical artery blood pH; 7 of the 10 patients in the study group with an artery blood base deficit of >10 mmol were in the subgroup with the persistently flat FHR traces (χ^2=10·07, P<0·01).

In one patient the screening record showed large decelerations with a pronounced loss of variation (mean range at 30 min of 18·9 ms) (Fig. 1). The patient had had one previous miscarriage and was in early labour (at a cervical dilatation of 2 cm). According to the study protocol the record, which should have lasted 60 min, was stopped at 30 min for an emergency caesarean section. The male infant weighed 3420 g at 41 weeks, required intubation, but had a 5-min Apgar score of 10. Measurements on the umbilical artery blood indicated a degree of hypoxaemia and acidaemia (pH 7·20, $P\text{CO}_2$ 51·9, $P\text{O}_2$ 7·9, base deficit 8·7 mM) given the early stage of labour. This was the only patient where the screening procedure affected immediate clinical management although there were possibly other, less immediate, effects. For the purposes of analysis this patient is included in the group screened for 60 min even though the screening record could not be completed.

The screening could not be done 'blind' and the attending midwife was aware of its duration and of the quality of the FHR pattern. The outcome of the study could therefore have been influenced unintentionally by changes of management caused by the procedure. This may account for the pattern of use of continuous FHR monitoring after the investigation was completed: 17 of 40 (42·5%) of those screened for 60 min compared with 156 of 548 (28·5%) of the remainder; but the difference was not significant. There was no difference in the use of monitoring in the groups screened for ≤30 min. However, it was expected that continuation of monitoring would be most likely in the subset of 14 patients with persistently low variation. This was observed: 9 of 14 (64%) were monitored continuously, after screening, until delivery, an excess compared with the 28·5% who were screened for ≤30 min (χ^2=6·82, P<0·01).

Of the group screened for 60 min, four were delivered operatively for fetal distress. One was the woman already described who had an imme-

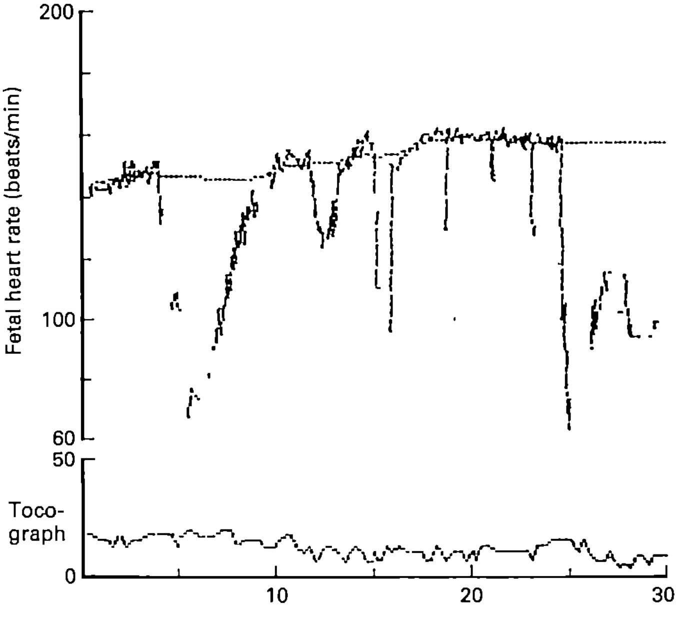

Fig. 1. Cardiotocograph from the one patient with a decelerative FHR trace of low variation. The recording was stopped after 30 min for delivery by caesarean section of a moderately acidaemic infant.

diate caesarean section. Of the other three, two were not monitored continuously after screening. The third was delivered at 40 weeks by forceps, with Apgar scores of 4 and 10 at 1 and 5 min respectively. The umbilical artery blood measurements were: pH 7·13, $P\text{CO}_2$ 46 mmHg, $P\text{O}_2$ 13 mmHg, and base deficit of 13 mM. Thus there was only one patient where intervention for fetal distress could have been influenced by the greater use of continuous FHR monitoring because of the screening procedure.

It is difficult to make an accurate diagnosis of fetal distress in labour. Of the 24 women whose labour was terminated operatively for this reason, 20 had umbilical artery blood gas measurements. The mean values (SD) were pH 7·19 (0·11), $P\text{CO}_2$ 51·4 mmHg (13·9) and $P\text{O}_2$ 11·6 mmHg (5·5). These were not significantly different from the mean values in the 331 women who were delivered spontaneously—pH 7·21 (0·09), $P\text{CO}_2$ 48·6 mmHg (10·6) and $P\text{O}_2$ 13·2 mmHg (6·3); or of the 74 women who were delivered operatively for other reasons—pH 7·23 (0·08), $P\text{CO}_2$ 48·4 mmHg (10·3), $P\text{O}_2$ 13·3 mmHg (7·1). Nine (45%) newborns, delivered for fetal distress, had a base deficit of 10 mM, a significant excess ($\chi^2=4{\cdot}62$, $P<0{\cdot}05$) compared with the rest of the group. This can be compared with 7 (70%) of the group identified, in early labour, by persistently low FHR variation, but most of the 99 infants with an artery blood base deficit of $\geq$10 mM were in neither group.

Discussion

Visual interpretation of FHR recordings allows grossly abnormal features, such as decelerations, to be detected but assessment of variation is more difficult. Overall there are inconsistencies between different observers at the same time and the same observers at different times (Trimbos & Keirse 1978; Lotgering *et al.* 1982; Jongsma & Eskes 1985). The use of computerized numerical analysis gives objective measurements. The system we have devised and described in previous reports (Lawson *et al.* 1984) works in real time from a standard monitoring system. The analyses are available immediately to guide clinical management.

With exact measurements of FHR variation it is possible for the first time to determine the range of normality, which is wider than was suspected previously (Dawes *et al.* 1982*b*). Unusual or abnormal patterns can be recognized consistently, which facilitates screening. We chose to screen for lack of variability and presence of decelerations on admission to the delivery suite. Variability is normally reduced during phases of quiet sleep that are randomly distributed in relation to the time of starting monitoring. An extended sample of recording of at least 60 min is needed to distinguish a fetus that is at the start of a long period of quiet sleep from one which has a real reduction in FHR variation. However, we restricted recordings of this duration to the 5% of patients with the least variable heart rates using the same technique as in a previous study for screening antenatally (Lawson *et al.* 1984).

Eight patients were found to have decelerations despite normal variability. Excluding one where the FHR pattern resulted from recording artefacts, in the other seven the decelerations were all variable in type and not surprisingly were associated with more advanced labour. There were no significant differences in outcome from the rest of the group, in particular with respect to the average birthweight and umbilical artery blood gas measurements. We concluded that fetuses with decelerations on a background of normal variation were not compromised. The patients identified with reduced variation at 60 min, presenting at an earlier gestational age, were more likely to be in spontaneous labour, less likely to have received oxytocin, but as a group had an average duration of labour after screening no different from the rest.

Unfortunately it was not possible to obtain a subjective fetal movement count during the screening procedure. It is known that fetal thoracic movements cease before spontaneous labour begins (Castle & Turnbull 1983). If there were a parallel reduction in all body movements this might be associated with a reduced FHR variation. However, the observations exclude the possibility that induction of labour might, of itself, preselect for, or cause, reduced variation and bias the results accordingly. The difference was apparently not caused by the stage of labour at which screening was undertaken or by whether or not the membranes were intact.

The analysis is complicated by the fact that the screening procedure appeared to influence the subsequent use of continuous FHR monitoring, which was more likely in those with the longest screening time. This could explain the significant trend to more operative delivery for 'fetal distress' with increasing screening time. Continuous monitoring is known not only to detect patterns reflecting fetal distress but also to cause inappropriate operative intervention unless fetal pH is checked directly from a scalp blood sample (Zalar & Quilligan 1979). Thus the outcome in terms of intervention for fetal distress may have been biased by the screening measurement. That this did not happen cannot be proved. However, it is unlikely for two reasons. Although there was a significant trend to more operative delivery for fetal distress as screening time increased, there was not a parallel trend for greater use of continuous FHR monitoring. Only in the 60-min group was there more continuous monitoring and this could have affected only one of the four subsequent operative deliveries for fetal distress; that is not enough to bias the results towards the trend observed. Secondly, a significantly higher proportion of the infants delivered for fetal distress were acidaemic compared with the rest. Thus the diagnosis of fetal distress was not inappropriate. The acidaemia was not the consequence of the operative delivery itself, because those similarly delivered for other reasons had normal umbilical artery blood gas measurements.

The sampling errors associated with the episodic nature of FHR variation led to the prediction that those selected for low variation after 30 min would fall into two groups. The FHR pattern of some, sampled at the beginning of an episode of low variation (quiet sleep), would regress back towards the mean in the next 30 min as the fetus changed normally to a phase of active sleep. This group would be expected to be more normal in its characteristics than the remainder in whom the low FHR variation persisted throughout the 60 min. This prediction was borne out. The latter group were slightly but significantly more preterm as a result of spontaneous labour, and were significantly more acidaemic at delivery.

Further studies are needed to investigate the possible clinical usefulness of FHR screening in early labour. The detection of decelerations on a background of normal FHR variation appears not to be useful. The detection of low FHR variation without decelerations identifies a group that is apparently at risk of acidaemia at delivery but (based on a small sample) appears to have an excellent outcome. However, it is possible that intervention (operative delivery) obscured the relation between the screening procedure and the outcome. The pattern of decelerations with low FHR variation is rare in an unselected population of women (about 0·2%) and is possibly the only pattern of clinical significance. Finally, an apparently normal FHR variation on admission in labour does not preclude a later diagnosis of fetal distress deemed to be bad enough to require operative delivery.

A similar result has been reported in a recent study of a 20-min screening record of the FHR on more than 1000 low-risk women in labour. The results of the screening were concealed. An 'ominous pattern' was observed in 10 women of whom one had a fetal death in labour and one was delivered operatively for fetal distress, that is, an immediate, poor outcome affected about 0·2% of the sample. Only four of the 34 women requiring operative delivery for fetal distress were detected by the screening procedure (Ingemarsson *et al.* 1986).

Our study does not establish whether screening is clinically helpful or not. But if such screening were to modify the management and outcome of only two per 1000 with benefit, a large controlled trial (of more than 10 000 patients) would be necessary to demonstrate that fact. It is not clear from our data whether or not it is clinically useful to identify low FHR variation without decelerations, which was associated with acidaemia at birth but not with a poor clinical outcome, possibly because of the ameliorating effects of more operative intervention for fetal distress.

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Fetal outcome in obstetric cholestasis

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Summary. Obstetric cholestasis has been associated with a high incidence of stillbirth and perinatal complications. Between 1975 and 1984, 83 pregnancies were complicated by cholestasis. Meconium staining occurred in 45%, spontaneous preterm labour in 44%, and intrapartum fetal distress in 22%. Of 86 infants two were stillborn and one died soon after birth. Perinatal mortality fell from 107 in a previous series from this hospital (1965–1974) to 35/1000 in this series. Cardiotocography, estimations of oestriol, liver function tests and ultrasonic assessment of amniotic fluid volume failed to predict fetal compromise, whereas amniocentesis revealed meconium in 8 of 26 pregnancies. Early intervention was indicated in 49 pregnancies, 12 because of fetal compromise. This study suggests that intensive fetal surveillance, including amniocentesis for meconium, and induction of labour at term or with a mature lecithin/sphyngomyelin ratio, may reduce the stillbirth rate in this 'high-risk' condition.

Obstetric cholestasis is a condition of pruritus with or without jaundice which begins in the latter half of pregnancy and resolves in the puerperium. Also known as intra-hepatic cholestasis of pregnancy, it is characterized by geographical variation, occurring commonly in Scandinavia and Chile (Haemmerli 1966; Rencoret & Aste 1973), occasionally in China and Australia (Zhong-Da *et al.* 1983; Kater & Mistilis 1967) and is rarely reported in other countries.

From the maternal viewpoint, it is essentially benign except that pruritus may become severe and generalized. An increased frequency of urinary tract infection and postpartum haemorrhage has been identified in some series (Rencoret & Aste 1973; Johnston & Baskett 1979).

Obstetric cholestasis was initially also considered benign from the fetal viewpoint (Orellana *et al.* 1961; Roszkowski & Miedzinska 1968). Subsequent series demonstrated preterm labour in 30–60% (Haemmerli 1966; Johnston & Baskett 1979). Additional risks were identified in a report from the Royal Prince Alfred Hospital in Sydney of 56 cases between 1965 and 1974, with meconium staining of the amniotic fluid in 27%, intrapartum fetal distress in 18% and perinatal mortality in 11% (Reid *et al.* 1976). In that series five fetuses died *in utero* and one in the first hour after birth from asphyxia.

The impact on fetal outcome of the recognition of cholestatic pregnancies as high risk was assessed by analysing all pregnancies with cholestasis in the 10 years following the 1965 to 1974 series.

Patients and methods

The case notes of all patients indexed as having obstetric cholestasis who gave birth between 1 January 1975 and 31 December 1984 at Royal

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Prince Alfred Hospital were reviewed; there were 89 pregnancies in 76 women. Four pregnancies were excluded as the notes suggested primary hepatic or dermatological aetiologies for the pruritus or jaundice. The diagnostic criteria described by Kater & Mistilis (1967) at this hospital were used until mid 1982 when the introduction of a total bile acid assay and hepatitis A Igm estimation permitted additional criteria of elevated serum bile acids and negative hepatitis serology. Two pregnancies with normal bile acid levels were excluded. The final series comprised 83 pregnancies complicated by obstetric cholestasis in 71 women, from 41 419 deliveries over the 10-year period (prevalence 0·2%).

Although obstetric management remained at the discretion of the individual consultant, antenatal monitoring and induction of labour were increasingly employed during the study period. The primary method of fetal monitoring was by urinary oestriol (E_3) estimation until 1979 and afterwards by non-stress cardiotocography (CTG). E_3 estimations after 1979 were performed on serum. Ultrasonic estimation of amniotic fluid volume was qualitative. Amniocentesis was favoured by some obstetricians as a means of detecting antepartum meconium staining. Fetal distress in labour was defined as a suboptimal fetal heart pattern (decelerations, bradycardia or tachycardia) necessitating operative delivery. Neonatal hyperbilirubinaemia was diagnosed when serum bilirubin exceeded 250 µmol/l in the first week of life, and hypoglycaemia when blood glucose fell below 1·5 mmol/l in the first 48 h. Respiratory distress syndrome (RDS) was defined as signs of respiratory distress with onset in the first 12 h of life and with characteristic lung radiographic changes.

Liver function tests were analysed by parametric statistics following log transformation. Perinatal mortality in New South Wales includes all stillbirths of ≥20 weeks gestation or 400 g and neonatal deaths within the first 28 days.

Results

Eighty-six infants, including three sets of diamnionic twins, were born from 83 pregnancies. Two stillbirths and one neonatal death gave a perinatal mortality rate of 35/1000. Compared with the earlier series, stillbirth and perinatal mortality rates fell in this series by 66/1000 (confidence intervals (CI) −9 to +139) and 71/1000 (CI −11 to +153) respectively.

The three perinatal deaths occurred at 35–36 weeks gestation. Thick meconium was found in the amniotic fluid in each. The two stillbirths occurred before labour and the neonatal death was due to asphyxia 4 h after birth by emergency caesarean section, with a cord artery pH of 6·70 after a preterminal CTG. Autopsies in two showed petechial and pericardial haemorrhages, consistent with acute intrauterine anoxia. The remaining necropsy was unremarkable. None had evidence of chronic intrauterine ischaemia. All three women had been hospitalized for monitoring, in two of them the E_3 was ≥10th centile 2 days beforehand, while two of them had a reactive CTG on the day before fetal death. Birthweight in each was >50th centile using local standards (Kitchen *et al.* 1983). Placental examination and histology was non-contributory apart from meconium staining of the amnion and cord.

Four patients in this series had a previous stillbirth (11% of multiparae), one in a pregnancy with cholestasis, one due to chronic villitis of unknown aetiology, and two in pregnancies complicated by pruritus in which cholestasis may have been present but the diagnosis was not made.

E_3 was estimated on 426 samples from 60 pregnancies, and 47 (11%) were below the 10th centile. Of the 428 CTGs performed in 46 pregnancies, only one was sufficiently abnormal to indicate delivery. Sixty-eight scans were performed in 38 pregnancies. Of the nine patients with oligohydramnios, two had meconium-stained amniotic fluid at delivery and two developed intrapartum fetal distress. Amniocentesis to detect meconium was performed on 41 occasions in 26 singleton pregnancies at a gestational range of 29–39 weeks. After demonstration of clear fluid, amniocentesis was repeated at weekly intervals once in two patients, four times in one patient, and seven times in another. Meconium staining was found in eight pregnancies (31%) at amniocentesis, in four of which gestational age was ≤32 weeks.

Intervention (induction or caesarean section) was indicated in 12 pregnancies (14%) with evidence of fetal compromise. Eight had meconium staining at amniocentesis, one a preterminal CTG, and three serial E_3 estimations <10th centile. Delivery resulted in successful outcome in all but the one woman with the abnormal CTG.

Intervention at term or after demonstration of mature lecithin/sphingomyelin ratio (L/S) occurred in a further 37 pregnancies (43%) without evidence of fetal compromise. The indication was solely 'high-risk' pregnancy.

Overall, intervention occurred in 49 pregnancies (59%) of which 16 were preterm. Neonatal morbidity in the preterm intervention group comprised RDS in four, hyperbilirubinaemia in four and hypoglycaemia in two. Only one of the four babies who developed RDS was from a pregnancy without evidence of fetal compromise.

Of 81 pregnancies resulting in a livebirth, 27 laboured spontaneously and in 36 labour was induced, 18 were delivered by caesarean section before labour started, while four of the spontaneous and eight of the induced labours eventually required abdominal delivery, giving a section rate of 37%. Twelve of the 27 spontaneous labours (44%) occurred <37 weeks. Intrapartum fetal distress necessitated delivery in 14 of 63 labours (22%).

Meconium staining was a feature of 37 of 83 pregnancies (45%). In addition to eight patients who had stained fluid at amniocentesis, meconium-stained amniotic fluid was noted in a further nine at amniotomy, in 15 at spontaneous rupture of the membranes, in one at caesarean section before labour, and in four in whom meconium followed intrapartum demonstration of clear amniotic fluid.

Figure 1 shows maternal liver function tests in pregnancies ending in perinatal death, compared with those which did not. Maternal liver function tests are compared in Fig. 2 for pregnancies with and without meconium, fetal distress and prelabour fetal compromise (meconium at amniocentesis, preterminal CTG, or serial E_3 <10th centile). As total bile acids were assayed in only 12 patients, statistical comparisons were not possible for mean bile acid levels except between pregnancies with meconium (27·4 µmol/l; CI 9·9 to 75·5) and with clear fluid (26·1 µmol/l; CI 9·3 to 73·3).

Discussion

Stillbirth and perinatal mortality rates declined by 66 and 71/1000 respectively in this series of patients with obstetric cholestasis compared with an earlier series (1965–1974) from the same hospital (Reid *et al.* 1976). Due to the rarity of cholestasis, and thus the small numbers involved, the CI indicate that the change fell short of significance at the 5% level. Despite a high rate of preterm delivery in both studies, improved neonatal care was not responsible for the fall in perinatal mortality as the perinatal losses in both series were mainly intrauterine deaths with both neonatal deaths occurring at gestations ≥36 weeks.

We propose two reasons for this improved fetal prognosis. Firstly, the recognition of cholestatic pregnancies as high risk resulted in induction of labour in 37 pregnancies without evidence of fetal compromise. Secondly, intensive fetal surveillance identified 12 pregnancies with antepartum fetal compromise such that intervention prevented adverse outcome in 11.

Reid *et al.* (1976) noted increased risks of intrauterine death, meconium, preterm labour, and fetal distress in the 1965–1974 series from this centre. Although not all authors accept that obstetric cholestasis is associated with adverse perinatal outcomes (Roszkowski & Miedzinska 1968; Ylostalo 1970; Johnson 1973), analysis of all cases documented in the English language literature (Table 1) yields meconium-stained amniotic fluid in 27%, intrapartum fetal distress in 18%, a stillbirth rate of 30 and a perinatal mortality rate of 70/1000. In this study, high rates of meconium staining (45%), preterm labour (44%), and intrapartum fetal distress (22%) were confirmed. Such frequency of meconium is unusual, especially as in over half (19 of 37) it was identified before 37 weeks, and often preceded labour, developing intrapartum in only four.

Following the earlier study, some obstetricians favoured induction of labour at 37 weeks as with other high-risk pregnancies. Success elsewhere is claimed with such policy (Ranta *et al.* 1979). As stillbirths may occur before term, Shaw *et al.* (1982) advocated induction once fetal lung maturity was established. In this study, 37 pregnancies at term, or with a mature L/S ratio were terminated solely on the basis of the diagnosis of obstetric cholestasis. There was a small but manageable morbidity in terms of RDS and jaundice attributable to the high level of intervention (59%).

Intervention was indicated in a further 12 pregnancies due to evidence of antepartum fetal compromise. The indication in three was consecutive E_3 estimations <10th centile. As only 11% were <10th centile and as the last E_3 in two of the women with perinatal deaths was ≥10th

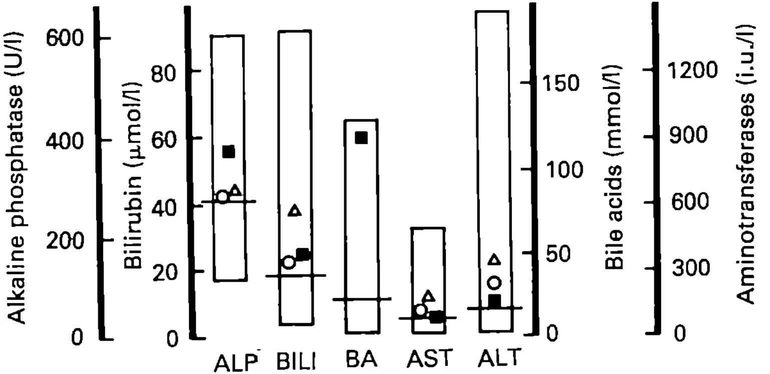

Fig. 1. Results of liver function tests in three pregnancies ending in perinatal death (2 stillbirths △ and ○, neonatal death ■) compared with geometric means ±2 SD calculated from pregnancies that resulted in surviving infants. ALP, Alkaline phosphatase; BILI, bilirubin; BA, bile acids; AST, aspartate aminotransferase; ALT, alanine aminotransferases.

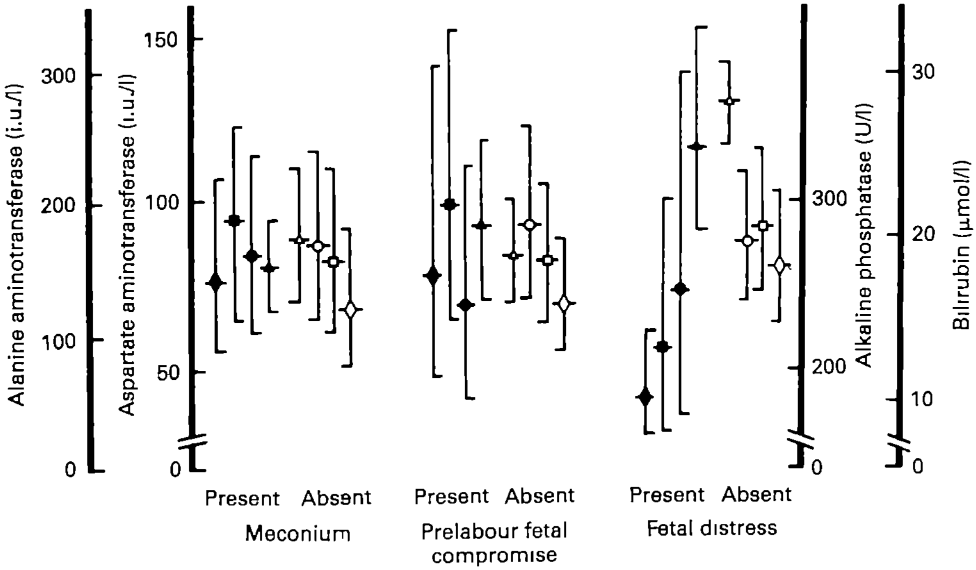

Fig. 2. Geometric means and 95% confidence intervals of maternal liver function in pregnancies with and without meconium, prelabour fetal compromise (meconium at amniocentesis, preterminal CTG or serial E_3 <10th centile) and fetal distress in labour. ◇, Alanine aminotransferase; □, aspartate aminotransferase; ○, bilirubin; △, alkaline phosphatase.

centile, these results support other studies which note the inadequacy of biochemical monitoring in predicting fetal compromise in obstetric cholestasis (Laatikainen & Ikonen 1975; Garoff 1976).

CTG identified only one patient with antepartum fetal compromise. A reactive pattern was followed the next day by a preterminal trace, and intervention occurred too late to prevent death from asphyxia. Others have also found CTGs poorly predictive in cholestasis (Kaar 1980). Laatikainen & Ikonen (1975) advocated the oxytocin challenge test without substantiating evidence, while microprocessor analysis of short-term CTG variability has proven less predictive than in other high-risk conditions (Ammala & Kariniemi 1981). In view of the failure of conventional tests to predict fetal compromise, and in view of the high frequency of meconium in obstetric cholestasis, antepartum monitoring of amniotic fluid has been tried. Using amnioscopy, Laatikainen & Ikonen (1977) found meconium in 8 of 88 patients, and Ylostalo & Ylikorkala (1975) reported a perinatal mortality rate of 19/1000. Amniocentesis allows direct scrutiny of amniotic

Table 1. Perinatal correlates of cholestasis

	Meconium		Intrapartum fetal distress		Stillbirth		Perinatal death	
Reference	No.	Patients	No.	Patients	No.	Births	No.	Births
Haemmerli (1966)	—	—	—	—	—	—	16	43
Friedlander & Osler (1967)	—	—	—	—	5	103	19	103
Roszkowski & Miedzinska (1968)	—	—	—	—	2	42	—	—
Ylostalo (1970)	—	—	—	—	1	43	—	—
Johnson (1973)	—	—	—	—	1	57	1	57
Laatikainen & Ikonen (1975)	33	116	21	116	4	125	8	125
Laatikainen (1975)	14	41	4	41	0	41	0	41
Ylostalo & Ylikorkala (1975)	—	—	—	—	2	107	2	107
Reid *et al.* (1976)	12	45	8	45	5	56	6	56
Garoff (1976)	28	59	18	84	2	84	3	84
Huisjes (1971)	—	—	—	—	1	12	1	12
Laatikainen & Ikonen (1977)	19	86	17	86	1	93	2	93
Johnston & Baskett (1979)	—	—	—	—	1	42	3	42
Wilson & Haverkamp (1979)	6	8	3	8	1	9	1	9
Ammala & Kariniemi (1981)	—	—	8	59	0	64	0	64
Shaw *et al.* (1982)	7	12	2	12	0	18	0	18
Zhong-Da *et al.* (1983)	—	—	—	—	4	22	4	22
Berg *et al.* (1986)	12	101	—	—	1	101	2	101
Totals	131	493	81	451	31	1019	68	977
Weighted means	= 26·6%		= 17·9%		= 30/1000		= 70/1000	

fluid, and in this study showed meconium in 31% of pregnancies so monitored. Amniocentesis for meconium staining was the most useful of the fetal surveillance tests, identifying 8 of 12 pregnancies with antenatal fetal compromise.

Mechanisms other than fetal distress have been suggested to explain the frequency of meconium (Wilson & Haverkamp 1979). In animal experiments, bile acids have a direct effect on colonic motility (Kirwan *et al.* 1975), although the occurrence of other signs of fetal distress in one-third of those with meconium (Laatikainen & Ikonen 1975) suggests that its presence is not simply due to stimulated gut motility. All perinatal deaths over the 20 years of the two studies were associated with meconium staining of the amniotic fluid.

The mechanism of stillbirth in cholestasis is unknown. The stillbirths in both Sydney series were unassociated with intrauterine growth retardation, and autopsies suggested an acute anoxic insult (Reid *et al.* 1976). Thus unlike other high-risk obstetric conditions, chronic utero-placental insufficiency would not appear aetiological. This may explain the insensitivity of conventional fetal monitoring.

Several toxins have been proposed as the cause of such an acute anoxic insult: bile acids (Laatikainen 1975), amino acids (Kuutti-Savolainen *et al.* 1979) and lipoprotein X (Johnson 1973). Bile acid injection in animals results in hepatocellular damage (Carey 1973). Characteristic increases in bile acids in serum, amniotic fluid, and umbilical cord samples, however, have not correlated with fetal compromise (Shaw *et al.* 1982; Heikkinen *et al.* 1980). A weak association has been suggested between elevated maternal liver function tests and fetal compromise (Laatikainen & Ikonen 1975). No association was seen in this study between raised liver function and fetal distress, prelabour fetal compromise or meconium (Fig. 2), although liver function tests in the three pregnancies ending in perinatal death were all above, yet within 2 SD of their respective geometric means. Non-specific placental changes described in cholestasis include villous oedema, maturation defects, and increased syncytial sprout formation (Laatikainen & Ikonen 1975; Ylostalo & Ylikorkala 1975). One study postulates electron dense bodies in the syncitium to be accumulations of lipoprotein X (Costoya *et al.* 1980). No specific features were seen here in the three placentas from pregnancies ending in perinatal death. Alterations in fetal steroid synthesis (Laatikainen 1975), maternal prolactin (Ranta *et al.* 1979) and placental protein 10 (Titinen *et al.* 1985), have not been studied for association with fetal outcome. One study, using ^{133}Xenon, noted a reduction of intervillous blood flow in cholestatic pregnancies (Kaar *et al.* 1980). Preliminary evidence suggests normal Doppler umbilical artery waveforms in obstetric cholestasis (D. Ellwood, personal communication).

Increased awareness of obstetric cholestasis may lead to overdiagnosis (Wilson & Haverkamp 1979), potentially a problem in this study before the introduction of the bile acid assay. Although overdiagnosis may have partly accounted for the fall in perinatal mortality, the high frequency of meconium-stained fluid and fetal distress suggests that such effect is minor. Furthermore, if half the fetuses with evidence of antepartum compromise had not been identified and thus died, the perinatal mortality rate would have been similar to that in the former series.

This study provides additional evidence that intensive fetal surveillance and a policy of induction at term, or with a mature L/S ratio, may reduce the stillbirth rate in obstetric cholestasis. Amniocentesis to detect meconium was the most sensitive test of fetal well-being, and has the added advantage of yielding amniotic fluid for analysis of fetal lung maturity. The rarity of obstetric cholestasis makes prospective assessment of any management policy in one centre difficult. We suggest that amniocentesis to detect meconium may have a role in the monitoring of patients with this condition and could be repeated weekly from 30 weeks until meconium or fetal lung maturity is evident.

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Fetomaternal macrotransfusion in the Yorkshire region. 1. Prevalence and obstetric factors

T. C. LI, D. R. BROMHAM, B. M. BALMER

Summary. The prevalence of fetomaternal macrotransfusion (FMMT), defined as fetomaternal haemorrhage (FMH) ≥10 ml, was calculated in a series of 15 724 postpartum Kleihauer tests recorded in six hospitals. The mean prevalence in this retrospective survey, the largest published to date, was 0·83% (SEM 0·22) but values varied according to the technique used to quantify the volume of FMH. A number of obstetric factors that have been suggested as influencing the occurrence and extent of FMMT were examined. The frequencies of these factors in cases with FMMT were compared with those in a control group without FMMT selected from the same population. Manual removal of the placenta (*P*<0·0001) and fetal distress in labour (*P*<0·0001) occurred significantly more frequently in the case group. We are unable to confirm previous reports that other obstetric factors may influence the occurrence and extent of FMMT.

Fetomaternal haemorrhage (FMH) may be classified according to the calculated volume of fetal blood passing into the maternal circulation. It is believed that the minimum amount of fetal blood required to cause rhesus isoimmunization is 0·1 ml (Levine 1944) but in 80% of all FMH detected, the leak is less than this (Bartsch 1972). Fetomaternal macrotransfusion (FMMT) or massive FMH is defined as a leak of ≥10 ml of fetal blood into the maternal circulation and this may have clinical consequences other than isoimmunization (Renaer *et al.* 1976; Fay 1983).

The prevalence of FMMT detected antepartum, from the three largest reported series, ranges from 0·14 to 0·26% (Dudok de Wit *et al.* 1968; Bartsch 1972; Helderweirt 1974). On the other hand, postpartum detection of FMMT is commoner, with reported prevalences between 0·6 and 1·1% which suggests that most of the FMMT detected postpartum arise during labour (Table 1). Although intrapartum FMH would seem to be the more common occurrence, a single postpartum Kleihauer test, as performed on rhesus-negative patients, will not exclude the possibility that fetomaternal bleeding had occurred antepartum.

FMMT has been shown to have certain obstetric associations. Although some occur spontaneously with no obvious obstetric cause or association, others are associated with amniocentesis, external cephalic version, abdominal trauma, placental abruption or tumour (Banninger & Schmid 1977; Fay 1983). Intrapartum FMMT may be associated with fetal distress, sinusoidal fetal heart tracing, stillbirth and uterine exploration in the third stage of labour (Renaer *et al.* 1976; Fay 1983; Bromham & Fay 1983).

The purpose of the present study was to investigate the prevalence of FMMT, diagnosed postpartum, and examine any associated obstetric factors which may influence the diagnosis of

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Table 1. The amount and frequency of fetomaternal macrotransfusion (FMMT)

Authors	No. of women examined	Frequency of FMMT			
		>10 ml	>15 ml	>25 ml	>50 ml
Bartsch (1972)	1507			0·2%	
Dudok de Wit *et al.* (1968)	662	1·1%			0·5%
Lloyd *et al.* (1980)	1206		1·0%		
Poulain & Houchet (1971)	5488	0·6%			0·1%
Schneider *et al.* (1973)	6667	1·0%			
Woodrow & Donohoe (1968)	2029		0·2%		
Zipursky (1971)	1372		0·6%		

FMMT and their possible usefulness as a screen for perinatal well-being. To do this we retrospectively surveyed a sample of the general obstetric population drawn from those who are all screened for FMH, namely rhesus-negative patients.

Patients and methods

This survey was conducted in 11 Yorkshire Regional Health Authority teaching and district general hospitals that were within a 50-mile radius from Leeds (Table 2).

All patients selected for study had rhesus-negative blood group, without rhesus antibody ever being detected, and gave birth to rhesus-positive infants. All of them had had a routine postpartum quantification of fetomaternal haemorrhage (FMH) using the Kleihauer acid elution test.

Prevalence

In six of these hospitals complete records of all Kleihauer tests done were kept in the haematology departments. The total number of postpartum Kleihauer tests done in each of these six hospitals, during the period 1976 to 1984 inclusive, was counted. Those performed antepartum, after an abortion, and repeat counts after an initial high result were excluded.

Five hospitals expressed results as numbers of fetal cells per 50 low-power fields and considered 500 fetal cells/50 low-power fields to be equivalent to a FMH of 10 ml. The remaining hospital (Staincliffe) gave results as a ratio of fetal to maternal red cells with 1:500 being equivalent to a FMH of 10 ml. Using the conversion factor for the hospital concerned the number of patients with FMMT was identified and expressed as a percentage of the total number of patients with postpartum Kleihauer tests.

Obstetric factors

Cases of FMMT (FMH ≥10 ml), from the 11 hospitals originally selected, were identified either from the Yorkshire Regional Blood Transfusion Service records or from the records of local transfusion laboratories or both. The results of these two methods of identifying cases (regional and hospital) are summarized in Table 2.

Wherever a case of FMMT was identified a search of the hospital records was made to confirm the availability of the case notes of that pregnancy for study. Reference was made to the local labour ward delivery record for the selection of control subjects. For each identified and available case, both the immediately preceding patient and the immediately subsequent rhesus-negative patient, whose partpartum Kleihauer test showed FMH <10 ml, were chosen as control subjects. In two instances cases of FMMT were consecutive without intervening suitable control subjects. Under that circumstance the two preceding and the two subsequent rhesus-negative patients without FMMT were chosen as the four controls for the two FMMT cases. Thus, there were two identified control subjects for each case of FMMT (Table 2).

Where available in hospital notes, the following data were extracted for each case and the corresponding two controls: (1) Maternal age (years), (2) parity (deliveries after 28 weeks gestation), (3) gestation at relevant delivery (completed weeks), (4) mode of delivery including third stage, (5) last antenatal maternal haemoglobin (g/dl), (6) the presence of fetal

Table 2. Cases of fetomaternal macrotransfusion (FMMT) and controls identified and available for study in 11 hospitals in the Yorkshire region with prevalence of FMMT among postpartum Kleihauer tests calculated from six hospitals

Hospital	Total no of women with postpartum Kleihauer test	Cases of FMMT identified from		FMMT (%)	Cases available for study *n* (%)	Total controls identified	Controls available for study *n* (%)
		Regional records	Regional and hospital records				
St James's University Hospital, Leeds	3456	4	14	0·41	11 (78·6)	22	22 (100)
Leeds General Infirmary	3538	22	24	0·65	24 (100)	48	48 (100)
Staincliffe Hospital, Dewsbury	2359	35	47	1·9*	44 (93·6)	88	82 (93·2)
Huddersfield Royal Infirmary	2523	20	25	0·99	10 (40)†	20	8 (40)†
Manygates Maternity Hospital, Wakefield	1657	7	9	0·54	9 (100)	18	16 (88·9)
Pontefract General Hospital	2191	11	13	0·59	13 (100)	26	25 (96·2)
Bradford Royal Infirmary	—	25	25	—	25 (100)	50	44 (88)
Halifax General Infirmary	—	10	10	—	10 (100)	20	19 (95)
Harrogate District Hospital	—	8	8	—	8 (100)	16	15 (93·8)
St Luke's Hospital, Bradford	—	6	6	—	6 (100)	12	7 (58·3)
York District General Hospital	—	19	19	—	19 (100)	38	36 (94·7)
Total	15 724	167	200	0·83	179 (89·5)	358	322 (89·9)

*Differs significantly from other units. See text

†Retrieval low because of local changes in medical records department.

distress (as diagnosed by the clinician supervising labour).

As an extension of the main survey the following data were also recorded for all cases and controls from four of the 11 hospitals studied (St James's University Hospital, Leeds General Infirmary, Staincliffe Hospital and Huddersfield Royal Infirmary. (1) Presence of hypertension (defined as blood pressure ≥130/90 mmHg), (2) presence of persistent proteinuria (associated with hospitalization), (3) antepartum haemorrhage, (4) total duration of labour, (5) duration of third stage, (6) induction of labour, (7) use of intravenous oxytocin.

This part of the survey included 88 cases and 160 controls. Fetal or maternal ABO groups were not considered in any part of the survey (see Discussion).

The results were analysed by conventional statistical methods which are indicated as appropriate. Comparison of the frequencies of the obstetric factors between the study and control groups was by contingency table analysis. Comparisons of mean estimated volumes of FMMT within the study group was by the two-sample *t*-test on log-converted data to allow for skewed distribution.

Results

Prevalence

The total number of women with postpartum Kleihauer tests done and the numbers and percentage of cases of FMMT in each of the six hospitals studied are tabulated in Table 2.

In a total of 15 724 Kleihauer tests recorded in the six hospitals there were 131 instances of FMMT giving an overall mean prevalence of 0·83% (SEM 0·23, range 0·41–1·9). Contingency table analysis (6 × 2) revealed highly significant variation in these prevalences (χ^2 = 51·44, P<0·00001). Inspection showed this to be due to the much higher calculated prevalence of 1·9% at Staincliffe (2 × 2 analysis compared with remaining units: χ^2 = 49·50, P<0·00001). Excluding Staincliffe the mean prevalence was 0·63% (SEM 0·10).

Obstetric factors

The numbers of women in the case and control groups are summarized in Table 2. The mean volume of FMMT in the cases was 38·1 ml (SEM 1·1). Within the cases the mean volumes of FMMT in the subgroups of women delivered by caesarean section and those having a vaginal operative delivery did not differ significantly from those in the case group as a whole. Age, parity, gestation and maternal haemoglobin levels (Table 3), frequency of methods of delivery (Table 4), hypertension, clinically significant proteinuria, antepartum haemorrhage, induction of labour or the use of intravenous oxytocin infusion (Table 5) did not differ significantly between the case and control groups.

Management of third stage

Whether analysed for the whole group or only for the subgroup of vaginal singleton births, the proportion of deliveries associated with manual removal of the placenta was significantly higher in the cases than in the controls (2 × 2 contingency table analyses; Table 4). However, among the cases, the mean volume of FMMT was significantly lower in the subgroup of deliveries associated with manual removal of the placenta (26·1, SEM 1·1 ml) than in the case group overall. Although there was no significant difference between the two groups in total duration of labour, the mean duration of the third stage of labour was significantly longer in the case group (12·2, SEM 2·4 min) than in the control group (5·5, SEM 0·3 min) (two-sample *t*-test; t=3·69, P<0·001).

Fetal distress and cardiotocography

Of the 501 case and control women reviewed in the survey, 20 were delivered by elective caesarean section. Of the remaining 481, six case notes did not allow comment on the presence or absence of fetal distress in labour. Analysis of the remaining data showed that the proportion of labours associated with fetal distress was significantly different between the case and control groups. However, the clinical manifestations of fetal distress did not differ significantly between the two groups (Table 6). The mean volume of FMMT in the 41 cases with fetal distress did not differ significantly from that in the case group overall. In none of the 179 cases of FMMT reviewed was a cardiotocographic (CTG) sinusoidal rhythm noted by the clinicians. Neither did any of the 150 CTG tracings available from cases or controls show this pattern.

Discussion

Prevalence

Our findings are in good agreement with previous reports (Table 1). Staincliffe Hospital used

Table 3. Age, parity, gestation and maternal haemoglobin in cases and controls

	Cases			Controls		
	n	Mean	(SEM)	*n*	Mean	(SEM)
Age (years)	179	26·4	(0·4)	322	26·5	(0·3)
Parity*	177	0·85	(0·07)	318	1·02	(0·07)
Gestation (weeks)	179	39·0	(0·2)	322	39·3	(0·1)
Haemoglobin (g/dl)	177	12·4	(0·1)	320	12·3	(0·1)

*Previous deliveries after 28 completed weeks gestation.

Contingency table analysis (3 × 2) of patients who had had 0, 1–4 or ≥5 previous deliveries did not show a significant difference between the cases and controls (χ^2 = 0·34, NS).

There were no significant differences between the two groups (two-sample *t*-test).

Table 4. Methods of delivery in cases and controls

	Cases	Controls
All deliveries	179	322
Twins	5 (2·8)	2 (0·6)
Singleton	174 (97·2)	320 (99·4)
Vaginal delivery	158 (88·3)	288 (89·4)
Caesarean section	21 (11·7)	34 (10·6)
Elective	8 (4·5)	17 (3·7)
In labour	13 (7·3)	22 (6·8)
Third stage		
Routine	158 (88·3)	283 (99·0)*
Manual removal	21 (11·7)	3 (1·0)*
Vaginal singletons	154	286
Cephalic	150 (97·4)	280 (97·9)
Breech	4 (2·6)	6 (2·1)
Normal	133 (86·4)	253 (88·5)
Operative	21 (13·6)	33 (11·5)
Third stage		
Routine	135 (87·7)	283 (99·0)†
Manual removal	19 (12·3)	3 (1·0)†

* $\chi^2 = 25\cdot7$; $P<0\cdot0001$, † $\chi^2 = 26\cdot9$; $P<0\cdot0001$ (2 × 2 contingency table analyses with Yates' correction) There were no other statistically significant differences.

Table 5. Characteristics of cases and controls in the extended study (see text)

	Cases (n = 88) n (%)	Controls (n = 160) n (%)
Hypertension	19 (22)	28 (18)
Proteinuria	5 (6)	3 (2)
Antepartum haemorrhage*	3 (3)	2* (1)
Induction of labour	27 (31)	43 (27)
Oxytocin infusion	34 (39)	56 (35)

* One control subject had a placenta praevia, the remainder were confirmed abruptions.

2 × 2 contingency table analysis with Yates' correction for each factor showed no significant differences between the two groups.

fetal: maternal cell count ratios to quantify the amount of FMH (Mollison 1972) but all other hospitals in the study measured the amount of fetomaternal bleeding by counting the fetal cells in standard film and optical field, as originally proposed by Woodrow *et al.* (1965). We suspect that the difference in the reported prevalence between Staincliffe Hospital (1·9%) and the other five hospitals (mean 0·63%) is related to different methods used to quantify the FMH. This problem of quantification has been discussed in depth by Mollison (1972).

In addition to counting methods there are other factors known to influence the results of a Kleihauer test. Fetomaternal rhesus and ABO blood group incompatibility result in lower counts (Devi *et al.* 1968) but only if there is a significant postpartum delay in sampling. As the sampling delay was not recorded in the case notes we have examined, we have assumed that maternal blood was sampled immediately after delivery as is standard clinical practice. The impact of incompatibility on the Kleihauer test would thus be minimal and tend if anything to lead to underestimation of the prevalence derived here. In addition, with our larger cohort studies, presented here and elsewhere (Li & Bromham 1988), any effect of ABO incompatibility will equilibrate between the study and control groups.

Our studies of obstetric factors suggest that a less accurate estimate of prevalence would be derived from the Regional Blood Transfusion Services records. This is because of incomplete returns of data from peripheral laboratories (Table 2).

Obstetric factors

We have investigated the association between FMMT and the various intrapartum obstetric factors that could have an influence on FMMT.

Table 6. Presence of fetal distress during labour and main features in its diagnoses

	Cases n (%)	Controls n (%)
(a) Presence of fetal distress during labour	(n = 168)	(n = 307)
No fetal distress	127 (76)	286 (93)*
Fetal distress present	41 (24)	21 (7)*
(b) Main feature in diagnosis of fetal distress	(n = 41)	(n = 21)
Thick meconium staining	11 (27)	5 (24)
Bradycardia (<100 beats/min)	18 (44)	10 (47)
Tachycardia (>180 beats/min)	3 (7)	1 (5)
Delayed deceleration	6 (15)	4 (19)
Other	3 (7)	1 (5)

(a) *2 × 2 contingency table analysis with Yates' correction; $\chi^2 = 28\cdot0$; $P<0\cdot0001$.

(b) 5 × 2 contingency table analysis; $\chi^2 = 0\cdot5$; NS.

Age and parity did not influence the occurrence of FMMT in this and previous studies (Mehta *et al.* 1979). Haemoglobinopathies may influence the results of Kleihauer estimations (Stropnicky *et al.* 1982; Krauss *et al.* 1983) but in the present study the case and control groups showed no difference in mean haemoglobin levels.

Hypertension and proteinuria

An association between pre-eclampsia and rhesus isoimmunization has been suggested (Knox *et al.* 1961; Knox 1968). However, Zipursky *et al.* (1963*a*), Devi *et al.* (1968), and Jones *et al.* (1969) were unable to find any association between FMH and pre-eclampsia although in the latter study significantly more hypertensive women had a FMH ≥0·25 ml. No FMMT was reported in these studies. We found no difference in the frequency of hypertension or proteinuria between the case and control groups.

Antepartum haemorrhage

Placental abruption has been described in association with FMMT, predominantly where accidental (Renaer *et al.* 1976) or iatrogenic (Banninger & Schmid 1977) abdominal trauma was involved. FMMT associated with abruption has more severe fetal consequences than similar volume loss without abruption (Fay 1983). Minchin & Bhoyroo (1970) suggested the use of a Kleihauer test as a prognostic guide in patients with placental abruption or antepartum haemorrhage of indeterminate origin, a FMH >0·5 ml indicating an increased risk of perinatal mortality and morbidity. Whereas it may be that FMMT or FMH in general is usefully considered in such patients we are unable to confirm that antepartum haemorrhage is a predisposing factor. There is a lack of good published evidence confirming an increased frequency of Rhesus isoimmunization subsequent to an antepartum haemorrhage in a previous pregnancy.

Labour

Montague & Krevans (1966) found that the frequency of a FMH >0·25 ml was higher after emergency caesarean section or caesarean hysterectomy in labour than after an elective procedure. This may be because of associated fetal distress, which we have shown to be significantly associated with FMMT and need not imply that labour *per se* promotes FMH. In our survey neither the total duration of labour nor the use of oxytocin infusion differed between the case and control groups suggesting that longer labour or more forceful contractions are not specifically related to an increased risk of FMH or FMMT. Lloyd *et al.* (1980) also found no increased use of oxytocin in patients with FMMT.

Method of delivery

With the exception of twins, which had a significant fourfold increased frequency of FMH, Mehta *et al.* (1979) were also unable to detect a statistically significant effect of method of delivery on the occurrence of FMH. However, Zipursky *et al.* (1963*a, b*), Jorgensen (1977), and Montague & Krevans (1966) found that caesarean section was associated with FMH. Larger blood leaks of >10 ml, classifiable as FMMT, are not identifiable in the data from these small-scale studies. Devi *et al.* (1968) found that FMH occurred more commonly after forceps delivery, twin delivery and emergency caesarean section. In a small case-control study, Lloyd *et al.* (1980) found no difference in the methods of delivery between 43 cases of FMMT and 45 control subjects. In our larger study we have confirmed that the methods of delivery do not differ between the case and control groups, neither were there any statistically significant differences in the mean volume of FMMT between the different methods of delivery subgroups.

Management of the third stage

Zipursky *et al.* (1963*a*) and Devi *et al.* (1968) reported the highest prevalence of FMH in deliveries associated with manual removal of the placenta. These previous observations are consistent with our findings that manual removal of the placenta was more common in the cases than in the controls. The significantly longer third stage of labour in the cases obviously relates to this. Although conditions such as morbid adherence of the placenta could be associated with both an increased frequency of retained placenta and an increased risk of FMMT, we find no evidence of such conditions in the present study. It therefore seems more likely that the manipulation itself promotes the FMH in these patients. Although manual removal of the placenta is associated with an increased frequency of FMMT, the mean volume of FMMT in this subgroup was lower than that in the case group overall. The fetal blood detected in the maternal circulation after manual removal of the placenta is not usually a direct loss to the fetal circulation and will not, therefore, be of clinical importance to the infant.

Fetal distress and cardiotocography in labour

FMMT has been described in association with fetal distress (Farabow *et al.* 1970; Fay 1983; Axelsson 1983). Renaer *et al.* (1976) pointed out the lack of published reports on fetal heart rate patterns at that time. Subsequently case reports of sinusoidal fetal heart rate in association with FMMT have been reviewed (Bromham & Fay 1983). Using the definition proposed by Katz *et al.* (1983) we were unable to identify any instances of sinusoidal fetal heart rate amongst cases or controls in this study and therefore we conclude that it is an uncommon, although recognized, intrapartum manifestation of FMMT. The patterns of fetal distress did not differ between the cases and controls in the present study but we confirm for the first time that fetal distress is more common in association with FMMT. It is not possible from our data to define cause and effect for this relation. It may be that fetoplacental insufficiency or anoxia predisposes to FMMT. Conversely, chronic or acute fetal blood loss is likely to cause intrapartum fetal distress. Further prospective studies are required to elucidate this question.

In conclusion, we find that the only statistically significant common factors associated with FMMT are manual removal of the placenta and fetal distress. The former is a mechanical problem of no clinical importance to the newborn whereas the latter indicates an adverse situation. The perinatal outcome in these two groups is discussed by Li & Bromham (1988).

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Fetomaternal macrotransfusion in the Yorkshire region. 2. Perinatal outcome

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Summary. The perinatal outcome associated with fetomaternal macrotransfusion (FMMT), defined as fetomaternal haemorrhage ≥10 ml, was investigated in a study group of 179 cases of FMMT and compared with the outcome in 322 controls selected from the same population. Perinatal mortality and morbidity occurred more frequently in the case group ($P<0{\cdot}01$). The mean Apgar score at 1 min and the cord haemoglobin level differed significantly between the case and control groups ($P<0{\cdot}01$) and showed a significant inverse correlation with the estimated volumes of FMMT ($P<0{\cdot}0001$). There was no significant difference in birthweight between the case and control groups. The mean estimated volumes of FMMT associated with perinatal mortality and with low cord haemoglobin (≤9·0 g/dl) were significantly higher than the mean volume in the case group as a whole, which suggests the possibility of adapting these observations to a screening procedure.

Fetomaternal macrotransfusion (FMMT) has been defined by previous investigators (Renaer *et al.* 1976; Fay 1983) as a leak of ≥10 ml of fetal blood into the maternal circulation. Although FMMT detected postpartum is not a common condition—Li *et al.* (1988) reported a prevalence of 0·8% in rhesus-negative patients delivered in 11 Yorkshire hospitals—it has been estimated to contribute to as much as 3% of perinatal mortality (Renaer *et al.* 1976; Bromham & Fay 1983).

Apart from the well-known consequences of rhesus isoimmunization, FMMT has been described in association with neonatal anaemia (Griffin 1969; Axelsson 1983), intrauterine growth retardation (Axelsson 1983), perinatal death (Griffin 1969; Fay 1983) and maternal transfusion reaction (Renaer *et al.* 1976).

The purpose of this cohort study is to describe the perinatal outcome associated with FMMT.

Patients and methods

The patients included in this study have been described previously by Li *et al.* (1988). They were selected from a series of rhesus-negative women delivered in 11 hospitals in Yorkshire between 1976 and 1984 who had a postpartum Kleihauer test for quantification of fetomaternal haemorrhage (FMH). The case notes of 179 women who had a FMMT (FMH≥10 ml) were identified (cases) and also those of 322 controls who had not had a FMMT, as described by Li *et al.* (1988).

For this study the following data were recorded: (1) Gestation at delivery (completed weeks), (2) birthweight, (3) birthweight corrected for gestation (Altman & Coles 1980), (4) Apgar scores at 1 and 5 min, (5) cord blood or neonatal haemoglobin, (6) perinatal death, (7) neonatal morbidity defined by an Apgar score of ≤4 at 1 min or a neonatal haemoglobin ≤9 g/dl

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or the presence of neurological deficit, (8) pathologists' reports for perinatal death.

The results obtained from the case and control groups were compared using either the two-sample *t*-test (gestation, birthweight and cord or neonatal haemoglobin) or Mann–Whitney *U*-test (Apgar scores). Comparison of mean estimated volumes of FMMT within the case group was by the two-sample *t*-test on log-converted data to allow for skewed distribution. Frequencies of perinatal mortality and neonatal morbidity were compared between the groups using contingency table analysis. Cord haemoglobin levels and Apgar scores at 1 min from both the case group and the combined case and control groups were correlated with both the calculated volume of FMH and the percentage loss from the fetal circulatory volume (%FCV). The %FCV was calculated on the assumption that the circulatory volume represents 8·5% of birthweight (Hinchliffe 1987). In addition, volumes of FMMT above the 50th, 75th and 90th centiles for the case group (Li *et al.* 1988) were considered as potential indicators for perinatal mortality, Apgar scores $\leqslant 4$ and cord or neonatal haemoglobin $\leqslant 9{\cdot}0$ g/dl occurring in the case group. Sensitivity, specificity and predictive values were calculated for these.

Results

The number of cases of FMMT and controls identified, and the number of these actually studied, in the 11 hospitals in the Yorkshire Region between 1976 and 1984, inclusive, have been reported elsewhere (Li *et al.* 1988). In summary there were 179 cases and 322 controls.

Birthweight

As reported previously the gestations at delivery did not differ significantly between the case and control groups. These data were used in this study to derive a correction for the birthweight according to gestation. Uncorrected birthweight did not differ significantly between the groups (Table 1). After correction for gestation at delivery the mean birthweight in the case group was 13·9 g (SEM 37·6) below the expected birthweight and in the control group it was 32·6 g (SEM 23·9) above the expected birthweight. The corrected birthweights did not differ significantly between the two groups. The frequency of a corrected birthweight >2 SD below that expected for gestation was 13 of 171 (7·6%) in the case group and 16 of 320 (5·0%) in the control group (2 × 2 contingency table analysis; χ^2 with Yates' correction 0·93; $P<0{\cdot}05$).

Apgar scores

The median Apgar score at 1 min was 8 (range 0–10) in the case group and 9 (range 0–10) in the control group. The difference between the two groups was significant using the Mann–Whitney *U*-test (*U* 23 502; $P<0{\cdot}01$). The frequency distribution of the 1-min Apgar score in the two groups is shown in Fig. 1. The 5-min Apgar score was recorded in fewer than 40% of all the case notes. Although most missing values could be extrapolated from the 1-min Apgar score and showed a significant difference between the two groups (*U* 24 469; $P<0{\cdot}01$), further analyses based on the 5-min Apgar scores were not performed. In the case group the mean estimated volume of FMMT for cases with a 1-min Apgar score $\leqslant 4$ did not differ significantly (two-sample *t*-test; log-converted data) from the mean volume in the case group as a whole (Fig. 2). For the combined case and control groups there were significant inverse correlations between the 1-min Apgar score and both the calculated volume of FMH ($n = 488$; $r = -0{\cdot}20$; $P<0{\cdot}0001$) and the %FCV ($n = 486$; $r = -0{\cdot}21$; $P<0{\cdot}0001$). The correlations remained significant if log converted data for the case group only were considered.

Table 1. Birthweights in the case and control groups

		Birthweight (g)	
Group	*n*	Observed	Observed–expected*
Cases	171	3219 (48)	−14 (38)
Controls	320	3302 (29)	33 (24)

*Corrected for gestation, birth rank and sex (Altman & Coles 1980).
Results are mean (SEM)

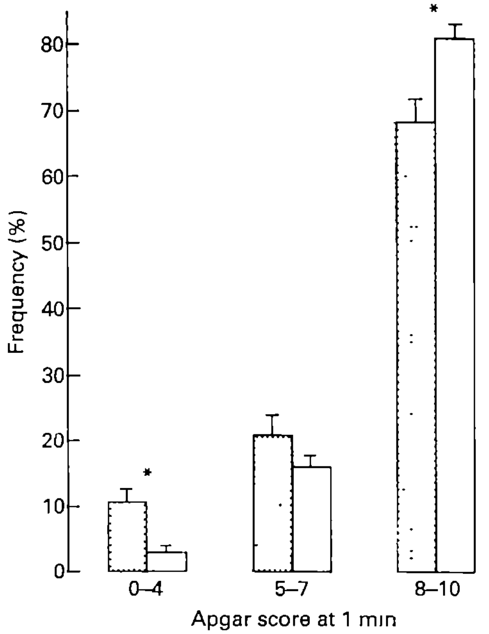

Fig. 1. Frequency (SE) distribution of Apgar scores at 1 min in the case (▨) and control (☐) groups. * Significant difference between the two groups ($P<0{\cdot}05$).

Cord blood and neonatal haemoglobin levels

A proportion of cord blood haemoglobin (Hb) results had not been recorded in the hospital notes because of technical difficulties (clotted samples etc.). Neonatal haemoglobin levels were obtained subsequently but because of the difficulty in applying universally meaningful correction factors for these, analysis was restricted to the available cord blood results. There were 358 of these in the two groups. The mean haemoglobin in the case group, 15·8 (SEM 0·3) g/dl, was significantly lower (two-sample t-test; t 3·26; $P<0{\cdot}005$) than that in the control group, 16·7 (SEM 0·1) g/dl. For the combined case and control groups there were significant inverse correlations between the cord blood Hb level and both the calculated volume of FMH ($n = 358$; $r = -0{\cdot}40$; $P<0{\cdot}0001$) and the %FCV ($n = 356$; $r = -0{\cdot}43$; $P<0{\cdot}0001$). The correlations remained significant if log-converted data for the case group only (Fig. 3) were considered. Of the infants whose cord blood Hb was known, 8 of 137 cases and none of 221 controls had a value of ≤9·0 g/dl (Fisher's exact test; $P = 0{\cdot}0008$). In the case group the mean estimated volume of FMMT for cases with cord blood Hb ≤9·0 g/dl was significantly different (two-sample t-test; log-converted data) from that in the case group as a whole (Fig. 2).

Perinatal mortality

There were 184 infants born to the 179 women in the case group and 324 to the 322 women in the control group. There were no perinatal deaths among the twin pregnancies. Of 10 perinatal deaths, eight occurred in the case group. There was also one death attributed to sudden infant death syndrome at 4 weeks in the case group; this baby was classified as a survivor for the purpose of this study. The explanations for these

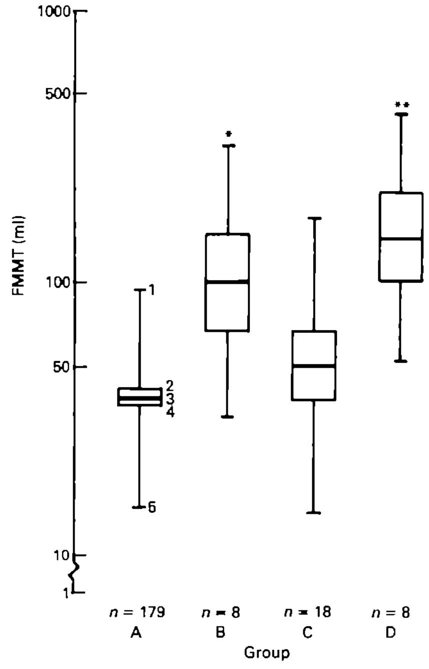

Fig. 2. Box-and-whisker graph illustrating the amount of fetomaternal macrotransfusion (FMMT) within subdivisions of the case group, according to perinatal outcome

Labelling convention for box-and-whisker: 1, geometric mean + SD; 2, geometric mean + SEM, 3, geometric mean; 4, geometric mean − SEM, 5, geometric mean − SD

A, Case group; B, perinatal mortality; C, Apgar score at 1 min ≤4; D, cord blood haemoglobin ≤9·0 g/dl

* Differs significantly ($t = 2{\cdot}33$, $P<0{\cdot}05$) from case group (A)

**Differs significantly ($t = 3{\cdot}62$, $P<0{\cdot}0005$) from case group (A).

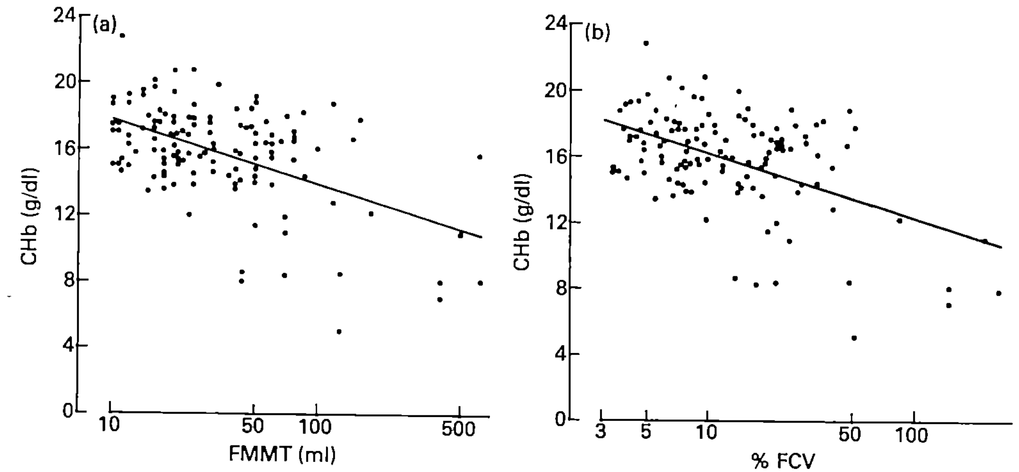

Fig. 3. Correlation between cord blood haemoglobin (CHb) and (a) volume of fetomaternal macrotransfusion (FMMT); $r = 0{\cdot}50$, $P<0{\cdot}00001$; and (b) percentage loss from fetal circulation (%FCV); $r = 0{\cdot}50$, $P<0{\cdot}00001$ (cases only).

deaths, where apparent from post-mortem examination recorded in the case notes, are summarized in Table 2. Of the eight deaths in the case group two had obvious causative factors other than FMMT. The remaining six perinatal deaths in this group were considered to have no other associated factor, although one infant with a cord blood Hb of 8·6 g/dl was an undiagnosed breech presentation and was born vaginally. Contingency table analysis (2×2, Fisher's exact test) showed a statistically significant difference between the two groups for the total frequency of perinatal death ($P = 0{\cdot}011$) and also for the frequency of perinatal death where no other causative factor was apparent ($P = 0{\cdot}004$). In the case group the mean estimated volume of FMMT associated with perinatal deaths was significantly different (two-sample *t*-test; log-converted data) from that in the case group as a whole (Fig. 2).

Neonatal morbidity

Using the definition given above, which was derived to include previously reported cases of neonatal morbidity associated with FMMT (Renaer *et al.* 1976; Fay 1983), it was possible to identify neonatal morbidity in 17 of the 176 sur-

Table 2. Perinatal deaths occurring in the case and control groups

Group	Gestation (weeks)	Birthweight (g)	Hb (g/dl)	FMMT (ml)	%FCV	Mode of death and post-mortem comment
Cases	39	3230	16·2	50	12·4	NND, hepatic necrosis, tyrosinaemia
	38	2700	NA	16	4·7	Fresh SB, exomphalos
	34	NA	NA	160	—	Fresh SB
	40	3980	8·6	44	88·4	NND (vaginal delivery, undiagnosed breech)
	34	3130	8·1	133	53·1	NND, hydrops unknown cause
	40	3620	NA	300	66·2	Fresh SB, 'fetus and placenta pale'
	40	4535	NA	600	105·8	Fresh SB
	40	3500	NA	80	28·6	Macerated SB
Controls	35	1500	20·6	—	—	SB, anencephaly
	32	1560	NA	—	—	SB, abruption

FMMT, Fetomaternal macrotransfusion; %FCV, percentage loss from fetal circulating volume; Hb, cord blood haemoglobin; NA, data not available from case notes; NND, early neonatal death; SB, stillbirth.

viving infants in the case group. A 1-min Apgar score of $\leqslant 4$ was found in 12 infants, haemoglobin $\leqslant 9{\cdot}0$ g/dl in four and neonatal convulsion in an additional case. The infant who died due to sudden death syndrome and a further infant who had recognizable delay in neurological development at 3 months, having been judged to be normal after birth and at 6 weeks, were not considered as having neonatal morbidity for this analysis. Among the 321 survivors in the control group there were seven with low 1-min Apgar scores and one with neonatal convulsions. Considering the surviving infants, the frequency of neonatal morbidity was 9·7% in the case group and 2·5% in the control group (2×2 contingency table analysis; χ^2 with Yates' correction 10·8; $P<0{\cdot}005$). Of the 19 infants with low Apgar scores, six of the 12 in the case group and all seven in the control group had other associated factors (maternal drug therapy in labour, instrumental delivery, preterm delivery). Excluding these 13 infants, the frequency of neonatal morbidity was 6·3% (11 of 176) in the case group and 0·3% (1 of 321) in the control group; contingency table analysis shows a significant difference between the two groups (Fisher's exact test; $P = 0{\cdot}0001$).

The specificity, sensitivity and predictive values of volumes of FMMT above the 50th, 75th and 90th centiles in the case group, for perinatal mortality, low 1-min Apgar scores ($\leqslant 4$) and cord or neonatal haemoglobin $\leqslant 9{\cdot}0$ g/dl occurring in the case group, are shown in Table 3. For example, FMMT >120 ml (>90th centile) has a sensitivity of 50% and a specificity of 91% for perinatal mortality. This implies that half of the perinatal losses would be detected at this level and that 91% of surviving infants would have volumes of FMMT below this range. The predictive value of a positive result is 20% suggesting that this proportion of perinatal deaths is associated with FMMT results in this range. Conversely, the predictive value of a negative result, the probability of surviving infants having a lesser volume of FMMT, is 97%. The equivalent indices for low Hb levels are even more encouraging.

Discussion

Birthweight

Axelsson (1983) reported a single case of FMMT in association with retarded fetal growth and fetal distress. Other possible cases appear in the series reported by Renaer *et al.* (1976), Fay (1983) and Bromham & Fay (1983). Although it could be postulated that a placenta 'failing' in one function, the maintenance of fetal growth, could also be defective in another, the haematological segregation of mother and fetus, there is no evidence in this study that low birthweight-for-gestation is significantly associated with FMMT. Studies of an alternative marker for FMH, alpha-fetoprotein, also prove disappointing for the prediction of low birthweight (Purdie *et al.* 1983; Hamilton *et al.* 1985; Tabor *et al.* 1987).

Table 3. Measures of efficacy for 50th, 75th and 90th centile levels of fetomaternal macrotransfusion (FMMT) in predicting perinatal outcome in the cases

	Cut-off centile for FMMT volume diagnostic for								
	Perinatal mortality (n = 179)			1-min Apgar score $\leqslant 4$ (n = 179)			Cord Hb $\leqslant 9{\cdot}0$ g/dl (n = 137)		
	50th (40 ml)	75th (70 ml)	90th (120 ml)	50th (40 ml)	75th (70 ml)	90th (120 ml)	50th (40 ml)	75th (70 ml)	90th (120 ml)
True positive	7	5	4	14	8	6	8	7	6
False positive	81	43	16	74	40	14	51	26	9
True negative	90	128	155	85	119	145	78	103	120
False negative	1	3	4	6	12	14	0	1	2
Sensitivity (%)	88	63	50	70	40	30	100	88	75
Specificity (%)	53	75	91	53	75	91	60	80	93
Predictive value of +ve result	8	10	20	16	17	30	14	21	40
Predictive value of −ve result	99	97	97	93	91	1	100	99	98

Apgar scores

Incomplete recording of Apgar scores at 5 min restricted us to consideration of the Apgar scores at 1 min. Our finding that FMMT was associated with significantly lower Apgar scores is in keeping with our previous report (Li *et al.* 1988) of a higher incidence of fetal distress in labour. The 'dose-response' correlation which we have noted strengthens this observation and even suggests a causal association.

Cord blood haemoglobin levels

We found significantly lower cord blood Hb levels in the cases than in the controls. However, it should be noted that although a subnormal cord Hb level is supportive evidence for FMMT, a normal level does not exclude it. The relation between neonatal anaemia and FMH has been discussed in depth by Goodall *et al.* (1958) and Pai *et al.* (1975). Acute intrapartum fetal bleeding could be associated with normal haematocrit if there were insufficient time for haemodilution to take place. A subnormal cord blood Hb therefore usually represents chronic loss or a bleed occurring several hours earlier. Chronic FMMT leading to severe fetal anaemia, in-utero congestive heart failure and associated hydrops fetalis has been reported and discussed by Renaer *et al.* (1976).

Perinatal mortality and morbidity

The importance of a Kleihauer test, to exclude FMMT, in all cases of apparently unexplained perinatal loss, irrespective of maternal rhesus status, has been emphasized (Renaer *et al.* 1976; Fay 1983). From our data, we can calculate the frequencies of perinatal mortality and morbidity associated with and attributable to FMMT (Table 4). In a large sample we found that the prevalence of FMMT was 0·83 per 100 deliveries (Li *et al.* 1988).

We can therefore calculate that FMMT is associated with perinatal mortality or morbidity in 1 of every 1282 deliveries. Van de Putte *et al.* (1972) reported a similar prevalence of 1 in every 834 pregnancies, although they used only neonatal anaemia to define morbidity. They further estimated that FMMT contributed to 3% of perinatal mortality in their population. More recent estimates made in the UK (Bromham & Fay 1983) broadly agree as does our finding of 1·9 to 2·5% in the present study (Table 4). Our finding might, in fact, be an underestimate as in some of the hospitals surveyed Kleihauer tests were not performed routinely after stillbirth even in rhesus-negative women. There were, therefore, at least two perinatal deaths, highly suggestive of associated FMMT, that did meet the inclusion criteria for these studies (Li *et al.* 1988). Renaer *et al.* (1976) reported one perinatal death and one case of neonatal anaemia in a prospective series unselected on the basis of rhesus group but selected by obstetric complication. Prospective data relating to FMMT in a totally unselected population are not available. More knowledge about this condition is therefore needed. If our assumptions, based on this sample of rhesus-negative women, are correct, FMMT may contribute to about 2% of perinatal mortality, comparable to conditions currently attracting considerably greater interest, e.g. obstetric infection or diabetes mellitus in pregnancy. Pregnancies affected by FMMT seem to have no major clinical markers (Li *et al.* 1988) and perinatal problems arise commonly in appropriately

Table 4. Perinatal mortality (PNM) and morbidity associated with and attributable to fetomaternal macrotransfusion (179 cases)

	n	% cases	Per 1000 deliveries*	% PNM†
Associated				
mortality	8	4·5	0·37	2·5
morbidity	17	9·5	0·78	—
Attributable				
mortality	6	3·4	0·28	1·9
morbidity	11	6·1	0·51	—

*Based on a 0·83% prevalence of FMMT (Li *et al.* 1988).

†Per cent PNM associated with or attributable to FMMT. Calculated from a PNM of 14·9 per 1000 in the Yorkshire region during 1980, the median year of study (MacFarlane & Mugford 1984).

grown normal babies. We report the conventional indices of efficacy of postpartum FMMT measurements in determining perinatal problems (Table 3), which compare favourably with equivalent values for physical and chemical assessments of fetal well-being (Whittle 1987; Westergaard *et al.* 1986). Prospective assessment of the value of antenatal and intrapartum screening of an unselected population, in conjunction with simplified haematological methods for detecting large volumes of FMMT, is also intended. At the very least, the ability to forewarn the neonatologist of the impending delivery of a grossly anaemic infant would be worth attaining. For the present we can only reiterate the importance of a Kleihauer test to assess the presence and degree of FMH in any case of unexpected perinatal death, neonatal anaemia or neonatal clinical problems compatible with recent major circulatory loss.

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The variation in cervical hydroxyproline and cervical water with age

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Summary. The squamocolumnar junction is frequently not visible in the postmenopausal patient. This study attempts to identify some of the changes in the cervix that may account for this observation. Twenty-four cervical biopsy samples taken at the squamocolumnar junction were analysed for hydroxyproline (collagen) and water content. There was significantly more hydroxyproline (collagen) in the premenopausal woman than the postmenopausal woman. Similarly, there was higher percentage of water in the biopsies in the premenopausal woman than the postmenopausal woman. Further samples obtained deeper in the cervical stroma did not confirm these differences. It appears likely that differences in cervical water and cervical collagen near the surface of the cervix account, at least in part, for the lack of visibility of the squamocolumnar junction in the older woman.

The success of cervical screening programmes in the UK has reduced the numbers of women dying from cervical cancer in the fifth and sixth decades (Macgregor & Teper 1978; Yule 1978). There has been less benefit to older women, who are less likely to avail themselves of cervical screening, and the screening process itself may be less effective. If a cytological abnormality is found in the postmenopausal woman the first diagnostic procedure may be a cone biopsy. Colposcopy, although still essential, is of less value in these older patients as often there is a failure to see the entire transformation zone. This raises the question of why the squamocolumnar junction recedes into the endocervical canal as a woman ages.

The cervix of the postmenopausal woman is smaller than that of the premenopausal woman, and the reduction of the cervical bulk tends to make the squamocolumnar junction recede into the canal (Crompton 1976). Eversion due to opening the bivalve speculum is less in the tight postmenopausal vagina, and may cause more distress or even trauma (Cartier 1977). Ostergard (1977) found the squamocolumnar junction in the canal of all patients over 60 years, and Paterson *et al.* (1982) could only see it in 6 of 12 of their postmenopausal patients. This study investigates the structure of cervical stroma in women of various ages, for two factors relevant to the relocation of the squamocolumnar junction with age.

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The water and collagen content near the squamocolumnar junction and deeper within the cervical stroma has been examined together with the possible influence of exogenous oestrogens on both these cervical components.

Collagen was assessed by assay of hydroxyproline, an amino acid that is virtually specific to collagen and variously reported as 10% (Kleissl *et al.* 1978) and 12% (Uldbjerg *et al.* 1983) of collagen. Collagen represents 82% of the total protein in the non-pregnant cervix (Danforth *et al.* 1974) and 85% of dry weight in the non-pregnant cervix (Uldbjerg *et al.* 1983). Acid-soluble collagen, on the other hand, accounts for only 28% of the non-pregnant cervix (Kleissl *et al.* 1978) and thus the majority of collagen in the non-pregnant state is old with acid stable cross-links (Uldbjerg *et al.* 1983). Measurement of the hydroxyproline content is an established method of quantification of collagen content (Danforth *et al.* 1960, 1974; Kleissl *et al.* 1978; Uldbjerg *et al.* 1983; Danforth & Buckingham 1973).

Patients and methods

Cervical biopsies were obtained from 46 women (age range 22–79 years) who attended the Western Infirmary, Glasgow. All the premenopausal women were attending either the gynaecological or colposcopy clinics with a variety of gynaecological complaints, but all had healthy cervices. The postmenopausal patients were recruited from women attending the menopausal clinics with climacteric symptoms. Provided the cervix was intact and healthy, there were no other inclusion criteria for the study. Written informed consent for cervical biopsy was obtained and approval was granted by the Western Infirmary ethics committee. All biopsies were obtained from healthy tissue; in 34 patients punch biopsy by Leech–Williamson forceps was obtained and the sample included a small piece of cervical epithelium. The biopsy was taken from the ectocervix, several millimetres from the external os. In the remaining 12 patients biopsy was obtained at hysterectomy from the cervical stroma, 0·5 cm deep to the epithelium at the squamocolumnar junction. No epithelium was present in these samples.

Biopsies were immediately frozen and stored at −20°C until analysed (maximum storage period 3 weeks). The tissue was dried at 37°C for 1 week and weighed to determine dry weight. After that 10 ml of 6N hydrochloric acid was added and incubated at 37°C for 1 week to break down the tissue. A 1-ml sample of this solution in 6N HCl was hydrolysed by heating at 105°C in an oil-filled heating block for 16 h and the hydroxyproline in the hydrolysate was assayed by the method of Blumenkranz & Asboe-Hansen (1974).

The first analysis was performed on punch biopsy specimens from 27 patients. Water content was calculated from wet and dry weights. Hydroxyproline was compared to dry weight to exclude age variation in cervical water. An association between hydroxyproline/dry weight ratio and percentage water with age and menopause was investigated.

There was an attempt to obtain as large punch biopsies as possible in this analysis. If the dry weight of biopsy was less than 8 mg then results were excluded, because at low weights the epithelial mass (with no hydroxyproline) was considered to be too great, relative to the stromal mass. The effect of too great an epithelial mass would be falsely to lower the hydroxyproline/dry weight ratio, but by exclusion of small biopsies this effect was reduced.

The same measurements were made on seven punch biopsies from women taking combined hormone replacement therapy, and 12 deep biopsies of cervical stroma obtained at hysterectomy for benign conditions other than uterovaginal prolapse. These samples were compared to the pre- and postmenopausal samples, both for water content and hydroxyproline content.

Results

In the first group analysed, despite attempts to obtain large punch biopsies, the range was 5·4–29 mg dry weight. Of the 27 samples three were excluded: two because they were ≤ 8 mg dry weight and one because clinical data could not be obtained; thus 24 samples remained in the analysis. The difference in cervical water content between the premenopausal (79·3%) and postmenopausal samples (75·9%) was statistically significant ($P<0·05$) (Table 1). This difference became apparent at the menopause and was not a gradual decrease in cervical water with age. Table 1 shows the hydroxyproline to dry weight ratio; this ratio is not dependent on cervical water so it is unaffected by these differences. Summating the ratios for the 13 premenopausal and for the 11 postmenopausal women showed a significant difference between

Table 1. Hydroxyproline (OHP) to dry weight ratio in cervical biopsies obtained from 24 women

Group	Age (years)	Weight of biopsy (mg) Wet	Weight of biopsy (mg) Dry	Water (%)	OHP/dry weight
Premenopausal	22	49·0	9·8	80·0	0·031
	23	70·0	14·6	79·2	0·034
	24	88·0	19·5	77·9	0·056
	25	141·0	29·0	79·2	0·068
	27	116·0	12·7	89·0	0·039
	30	60·5	20·0	67·0	0·055
	31	125·0	24·2	80·6	0·050
	31	41·8	8·8	79·0	0·068
	32	109·2	18·2	83·3	0·055
	37	96·4	19·4	79·9	0·067
	37	135·0	27·6	78·1	0·058
	44	74·8	16·4	78·1	0·049
	45	114·0	23·4	79·5	0·085
Mean				79·3	0·055
(SD)				(4·7)	(0·015)
Postmenopausal	43	50·8	10·5	79·3	0·048
	49	85·8	18·9	78·0	0·069
	50	90·6	21·2	76·6	0·099
	53	108·2	27·6	74·5	0·080
	56	98·6	25·1	74·5	0·100
	60	73·1	17·8	75·6	0·079
	61	64·1	15·6	76·6	0·087
	62	89·5	22·5	74·9	0·084
	67	100·0	24·2	75·8	0·066
	69	62·8	16·1	74·4	0·087
	79	47·5	11·8	75·2	0·085
Mean				75·9*	0·080**
(SD)				(2·2)	(0·015)

Significance of difference between premenopausal and postmenopausal means *$P<0{\cdot}05$, $t = 2{\cdot}2$; ** $P<0{\cdot}001$, $t = 4{\cdot}12$

these two groups (premenopausal 0·055, postmenopausal 0·080; $t = 4{\cdot}12$, $P<0{\cdot}001$, Student's *t*-test). The correlation coefficient of age to hydroxyproline content was significant ($r = 0{\cdot}74$, $t = 5{\cdot}20$, $P<0{\cdot}001$). As the age increased so did the hydroxyproline content indicating greater collagen density at the squamocolumnar junction with advancing age.

The second group of samples analysed included 12 biopsies taken from the deep cervical stroma and seven punch biopsies from women taking hormone replacement therapy. Seven of the deep biopsies were measured dry and wet and the cervical water was found to be considerably lower in the deep biopsies than in the superficial biopsies, and there was no decrease in water content with age. Similarly there was little variation in the hydroxyproline content with age in the deeper biopsies (Table 2). In general these biopsies had a hydroxyproline content similar to that found in superficial biopsies in postmenopausal women. Cervical water near the epithelium therefore appears variable and is dependent on age but deeper in the cervix there is a collagenous core with a water content that is not variable with age.

The samples from patients taking HRT included three small biopsies (Table 2). However, despite this the hydroxyproline to dry weight ratios remained high and were in the range of the postmenopausal patient and above the values found in premenopausal women. The water content, however, appeared higher than in postmenopausal women not taking hormone replacement therapy.

Discussion

The aim of this study was to account at least in part for the change in appearance of the cervix

Table 2. Hydroxyproline (OHP) to dry weight ratio and cervical water in 12 deep biopsies and in seven from women taking hormone replacement therapy (HRT)

	Age (years)	Weight of biopsy (mg)		Water (%)	OHP/dry weight
		Wet	Dry		
Deep biopsies	26	23·7	7·6	67·9	0·092
	27	NA	14·4	NA	0·083
	32	17·5	6·5	62·9	0·092
	40	NA	19·5	NA	0 052
	43	60·5	16·0	73·6	0·075
	46	NA	37·8	NA	0·083
	47	NA	14·7	NA	0·097
	59	64·0	18·3	71·7	0·066
	60	NA	21·3	NA	0·076
	64	94·7	27·6	70·9	0·065
	72	25·3	8·1	68·0	0·086
	76	46·1	10·3	77·7	0·068
Mean					0·078
(SD)					(0·013)
HRT	45	20·1*	4·4	78·1	0·091
	50	122·0	26·6	78·2	0·083
	51	66·1	15·2	77·0	0·066
	51	30·3*	6·2	79·5	0·065
	52	118·2	25·9	78·1	0·069
	53	19·6*	4·2	78·6	0·071
	63	42·5	11·8	72·2	0·085

* Small biopsies.
NA, Data not available.

with ageing. The most important change from the clinical viewpoint is the inability to demonstrate colposcopically the squamocolumnar junction in the older woman. Biopsies taken from this area have shown differences in percentage cervical water at the squamocolumnar junction between women of different ages, a change which appears to occur abruptly at the time of the menopause and is not gradual. The diminution of ovarian steroids with their water-retaining effect may be responsible for this. The effect of such a change will be to make the cervix more dense and less amenable to manipulation during colposcopic examination of the endocervix. Deeper in the cervical stroma there is reduced water, and little decrease with age is apparent.

The cervical water content found in this study is slightly higher than the findings of other authors. Kleissl *et al.* (1978) found 73% water in cervical biopsies taken at hysterectomy in five women aged 29–41 years. Danforth *et al.* (1974) found 74·4% cervical water in non-pregnant subjects but both these studies used full thickness blocks obtained at hysterectomy. Such values are in agreement with the data obtained in the deep biopsy group in this study. One study which obtained cervical tissue by rotating needle biopsy of cervix, a technique yielding similar tissue to the present punch biopsy study, found 80·8% cervical water in premenopausal women (Uldbjerg *et al.* 1983). These values are closely in accord with the data in this study for the premenopausal woman. Comparison of the values for postmenopausal women is not available, although animal models indicate that there is an increased water loss from connective tissue with age (Elden 1965).

These observations on cervical water explain some of the clinical findings. The cervix in the premenopausal woman is more oedematous (Cartier 1977) and in the subepithelial layer has an abundant blood supply, both of which regress after the menopause. These changes appear confined to the stroma near the surface epithelium but the cervical stromal core appears similar irrespective of age.

As the water content of the cervical stroma at the squamocolumnar junction decreases with age the hydroxyproline concentration rises. This is independent of the water content as the ratio to dry weight confirms. This change is more gradual and not as dependent on the menopause. The young patient has a thicker epithelium which with age gradually thins, especially after the menopause (Semmens *et al.* 1985; Marks & Shahrad 1977). The biopsies included the epithelium and so this must have some effect. By considering only biopsies of a reasonable size this effect is minimized. The change in the hydroxyproline content is real, not merely apparent, but it only affects the stroma adjacent to the epithelium. Deeper biopsies (Table 2) show no difference with ageing, and the hydroxyproline values are similar to those biopsies taken from the postmenopausal patients by punch biopsy.

Study of the data available on the anatomy of the cervix does not readily explain these findings. Hughesdon (1952), in a monograph compiled from histological study, firmly believed in a peripheral layer of smooth muscle which was continuous with the myometrium and the smooth muscle of the vagina. He believed this to be a functional layer and relevant to the process of cervical dilatation. Danforth (1954), although accepting the existence of a peripheral layer of smooth muscle, took a different view, believing that dilatation was due to the breakdown of cervical collagen and the excretion of hydroxyproline (Danforth & Buckingham 1973; Danforth *et al.* 1974).

The findings reported here are compatible with both views. Collagen content per weight is a reflection of collagen density. This will be increased if there is a greater amount of collagen or if there is a decrease in the other constituents of cervical stroma. The presence of smooth muscle will decrease collagen density. If this is found round the periphery of the cervix it will have the effect of producing lower hydroxyproline values in the superficial samples obtained from young women. After the menopause there are fewer nerves found in the cervix (Rodin & Moghissi 1973) and it seems reasonable to presume there will be reduced smooth muscle. This would increase the collagen density without an increase in total collagen.

Oestrogen will soften the 'pregnant' cervix by breakdown of collagen, especially in labour (Gordon & Calder 1977; Maillot & Zimmerman 1976), and during the reproductive years collagen is being produced and broken down continuously. After the menopause, when the oestrogen stimulus is considerably reduced, the collagen is likely to be more stable with more cross-linkages and consequently a greater proportion of insoluble collagen. The collagen content in the deep cervical stroma appears little influenced by advancing age. The findings on the patients receiving hormone replacement therapy suggest that the water content is similar to that in premenopausal women, probably indicating the fluid-retaining properties of the ovarian steroids. The collagen content is unaffected by hormone replacement therapy which has been shown to increase the visibility of the squamocolumnar junction in postmenopausal women (Roberts *et al.* 1986), and it may be that this tissue oedema induced by ovarian steroids aids manoeuverability and malleability of the cervix without a significant contribution from a decrease in collagen content.

Recent work has suggested that hormone replacement therapy will increase total skin thickness collagen content (Brincat *et al.* 1985). This suggests that oestrogen is responsible for synthesis of collagen and possibly also the decrease in collagenolysis in the skin. The current study has not investigated the total collagen content of the cervix and certainly the postmenopausal patient usually has a considerably smaller cervix than the premenopausal woman. The total collagen content of the cervix may be lower in the postmenopausal patient and consequently hormone replacement therapy may indeed increase the total collagen content in the cervix. But this study indicates that it does not increase collagen density.

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Endometrioid carcinoma of the ovary: a retrospective study

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Summary. Thirty-nine women with endometrioid carcinoma of the ovary were studied for factors relating to their presentation, treatment and survival. Nine of the women (23%) had a concomitant adenocarcinoma of the endometrium at the time of presentation. There were no significant differences in age at presentation or menopausal status between the two groups. The actuarial 5-year survival for women with endometrioid carcinoma of the ovary alone was 43%, 95% confidence interval (CI) 24% to 62% (stage I 80%, CI 45% to 100%, stage II 62%, CI 27% to 97%, and stage III 21%, CI 0% to 42%). The 5-year survival for women with concomitant endometrial tumours was 62·5% (CI 28·5% to 96·5%). The difference in the overall survival rate between the two groups was not significant.

Despite recent developments in chemotherapy for advanced carcinoma of the ovary, there has been little change in the overall prognosis for the disease, which still accounts for the deaths of over 3900 women per year in England and Wales (OPCS 1985). Factors which influence the prognosis for women with ovarian carcinoma therefore assume an important role in the management of the disease. It is known that prognosis varies according to the cell type of the tumour; even when matched for the stage of disease at presentation, the 5-year survival rate for serous cystadenocarcinoma is 26% compared with 62% for mucinous cystadenocarcinoma (Decker *et al.* 1973).

Endometrioid carcinoma of the ovary is the third most common epithelial tumour of the ovary, accounting for between 16% and 25% of these tumours (Long & Taylor 1964). Women with endometrioid ovarian tumours have a relatively favourable prognosis with a 5-year survival rate varying between 40% and 70% (Czernobilsky *et al.* 1970; Long & Taylor 1964). A coexisting adenocarcinoma of the endometrium has been found in 14–25% of patients with endometrioid carcinomas of the ovary (Czernobilsky *et al.* 1970; Klemi & Gronroos 1979). Women with these coexisting tumours are atypical since they are on average younger at presentation than patients with ovarian or endometrial carcinoma and have a better prognosis although these findings have not been subjected to statistical analysis (Czernobilsky *et al.* 1970; Klemi & Gronroos 1979; Eifel *et al.* 1982). The aim of this study was to determine whether the presence of a coexisting endometrial adenocarcinoma had a significant effect on the natural history or the prognosis for women with endometrioid carcinoma of the ovary.

Patients and methods

We have examined the case records of women who presented with endometrioid carcinoma of the ovary to the Samaritan Hospital for Women

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between 1970 and 1986 for information regarding age, parity, presenting complaints, histology and treatment. If the patient was dead, then details from the death certificate and post-mortem, if performed, were available. Patients lost to follow-up were traced through their general practitioner who reported the last time the patient was seen and their current state of health.

Statistics

Comparison of age at presentation in the two groups used the Mann–Whitney *U*-test since the distribution of age at presentation was unknown. Actuarial survival was calculated as not all women had completed a 5-year follow-up period. All calculations were made using a standard statistical computer package (SPSSX), and comparison between the rates of survival was performed by Lee–Desu analysis.

Results

During the period 1970 to 1986, 236 women presented with ovarian carcinoma. Of these 40 had endometrioid carcinoma of the ovary and full documentation was available for 39. Nine of these women were found to have a coexisting adenocarcinoma of the endometrium.

Most of the 30 women with endometrioid carcinoma of the ovary alone presented with either lower abdominal pain and swelling (60%) or postmenopausal bleeding (23%). The remaining women presented with either menorrhagia, irregular vaginal bleeding, stress incontinence, bilateral leg oedema or with an asymptomatic ovarian cyst. The average age at presentation was 54·7 years (range 37–76 years), 12 (40%) of the women were postmenopausal and 15 (50%) were nulliparous. In five women the endometrium contained areas of atypical hyperplasia, but only one woman had received unopposed oestrogens before presentation. Seven women, including three who were postmenopausal, had histological evidence of endometriosis.

The distribution by stage at presentation is shown in Table 1. There was bilateral ovarian involvement in 47% of the patients. A comparison of the histological grade of the tumours with the stage of disease at presentation showed that early disease was more frequently associated with well-differentiated grading whereas women with advanced disease were more likely to have poorly differentiated neoplasms (Table 1).

Primary treatment in all except one woman was surgical. Overall, 23 women underwent optimal pelvic clearance which included an infra colic omentectomy in seven. The number of patients receiving postoperative hormonal therapy, chemotherapy or radiotherapy varied according to the stage of disease at presentation. Of the patients with stage I disease, stage II and stage III disease, respectively, 40%, 77% and 87% received further therapy (Table 2). A wide variety of progestogens and chemotherapeutic agents was used during the study period. The use of progestogens was not dependent on the menopausal status of the patient or related to evidence of abnormal oestrogen stimulation. External beam irradiation was given to the pelvis using a three field plan, with a total dosage between 3·5 Gy and 5·3 Gy. Six women received further treatment for recurrent disease.

Seven (77%) of the nine women with concomitant endometrioid carcinoma of the ovary and adenocarcinoma of the endometrium presented with postmenopausal bleeding. One

Table 1. Stage at presentation and degree of histological differentiation of tumours in 30 women with endometrioid carcinoma of the ovary

Stage of disease	No. of patients	Degree of differentiation Well	Moderate	Poor
Ia	3	3	—	—
Ib	2	2	—	—
Ic	—	—	—	—
IIa	4	1	1	2
IIb	2	1	—	1
IIc	3	1	1	1
III	16	5	3	8

Table 2. Treatment of 30 women with endometrioid carcinoma of the ovary

Stage of disease	Surgery		Adjuvant therapy		
	Suboptimal	Optimal	Radiotherapy	Chemotherapy	Hormonal
Ia	1	2	1	—	—
Ib	—	2	—	—	1
Ic	—	—	—	—	—
IIa	—	4	2*	—	—
IIb	—	2	2†	—	—
IIc	1	2	3‡	—	—
III	5	11	5§	6‖	3¶

* One patient received progestogens in addition to radiotherapy.

† Both patients received progestogens in addition to radiotherapy.

‡ Two patients subsequently received chemotherapy but not for recurrent disease. One patient received progestogens in addition to radiotherapy.

§ Three patients received progestogens in addition to radiotherapy. Three patients subsequently received chemotherapy for recurrent disease, neither had undergone optimal surgery. One patient received radiotherapy followed by chemotherapy as primary treatment.

‖ Two patients received further chemotherapy for recurrent disease.

¶ One patient required chemotherapy for recurrent disease.

woman presented with irregular vaginal bleeding and the other with an asymptomatic ovarian cyst. The average age at presentation was 62·5 years (range 41–76 years), eight women (89%) were postmenopausal and five (55%) were nulliparous.

The correct staging of these concomitant tumours is difficult, but, when staged for the ovarian lesion alone one woman presented with stage I disease, two with stage II and six with stage III. The histological grade of both ovarian and endometrial tumours was similar in six of the cases. All nine women underwent optimal pelvic clearance, four also had an infra colic omentectomy (Table 3).

Eight of the women with concomitant tumours received adjuvant therapy and two of these received additional treatment, within 2 years of diagnosis, for recurrent disease.

The actuarial 5-year survival rates for the 30 women with endometrioid carcinoma of the ovary alone, by stage of disease at presentation, are shown in Fig. 1. The overall 5-year survival rates for women with either endometrioid carcinoma of the ovary alone or with concomitant endometrial tumours were 43% (95% CI 24% to 62%) and 62·5% (95% CI 28·5% to 96·5% respectively) (Fig. 2). The 5-year survival rate for a subgroup of women with endometrioid carcinoma of the ovary and atypical hyperplasia of

Table 3. Treatment of nine women with endometrioid carcinoma of the ovary and concomitant adenocarcinoma of the endometrium

Stage of disease (ovarian lesion)	Surgery		Adjuvant therapy		
	Suboptimal	Optimal	Radiotherapy	Chemotherapy	Hormonal
Ia	—	1	1	—	—
Ib	—	—	—	—	—
Ic	—	—	—	—	—
IIa	—	—	—	—	—
IIb	—	—	—	—	—
IIc	—	2	1	—	1
III	—	6	2*	3†	—

* Both patients received progestogens in addition to radiotherapy.

† One patient received progestogens in addition to chemotherapy. Two patients subsequently required radiotherapy for recurrent disease.

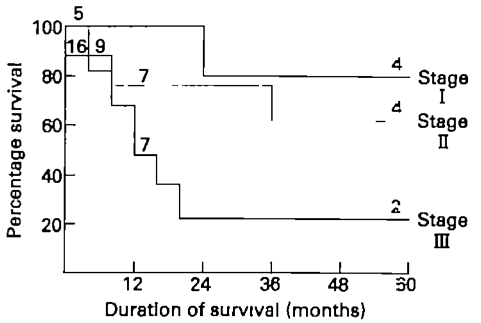

Fig. 1. Actuarial survival for women with endometrioid carcinoma of the ovary by stage at presentation. (Numbers on graphs refer to the women at risk during that time interval.)

the endometrium was 53% (95% CI 0 to 100%). The difference in the survival rates between the groups was not statistically significant.

Discussion

There was histological evidence of endometriosis in seven women with endometrioid carcinoma of the ovary, but endometriosis and endometrioid carcinoma were only found to coexist in the same ovary in two women. Cummins *et al.* (1979) have reported that only 25% of endometrioid carcinomas developed in areas of endometriosis. Although the frequency of endometriosis was higher in this study than previously reported it remains unlikely that endometriosis is the major predisposing factor in the development of ovarian edometrioid carcinoma (Kurman & Craig 1972; Ulbright & Roth 1985).

The prognosis for women with endometrioid carcinoma of the ovary has been reported to be the most favourable of the epithelial tumours (Long & Taylor 1964; Czernobilsky *et al.* 1970), but the overall survival rate for the women studied with endometrioid carcinoma, while being superior to that of serous cystadenocarcinoma, is inferior to mucinous cystadenocarcinoma. The stage of disease at presentation affects the survival rate for women with endometrioid carcinoma as it does for all other ovarian tumours (Einhorn *et al.* 1985).

The presence of a coexisting adenocarcinoma of the endometrium with endometrioid carcinoma of the ovary is a relatively common finding and was found in 23% in the present study. Earlier studies have reported that women with concomitant tumours presented at an earlier age than expected for either ovarian or endometrial carcinoma (Czernobilsky *et al.* 1970; Eifel *et al.* 1982). We found no statistically significant difference in age or menopausal status between the woman who presented with an ovarian lesion only and those who presented with concomitant tumours. It is not known whether endometrioid carcinoma of the ovary with a concomitant adenocarcinoma of the endometrium represents two independent primaries or metastatic disease. Evidence suggesting an independent origin has been based on the observation that the survival rates for patients with concomitant tumours were superior to the survival rates for either stage III endometrial carcinoma or stage II ovarian carcinoma (Scully 1977; Eifel *et al.* 1982). Using arbitrary criteria to assess the independent or metastatic nature of concomitant ovarian and endometrial tumours, Ulbright & Roth (1985) showed that concomitant endometrioid carcinoma of the ovary and adenocarcinoma of the endometrium, if moderately or well differentiated, were possibly independent in origin, whereas the poorly differentiated were possibly metastatic. In our study the histology of the concomitant tumours was similar in six patients suggesting that the lesions were metastatic in nature, probably arising from the ovary, since there was no evidence of atypical hyperplasia in the endometrium surrounding the adenocarcinoma.

The presence of a concomitant endometrial carcinoma was not detrimental to the prognosis of the patient. Life survival analysis shows that the difference in the survival rate between women with concomitant tumours and those with endometrioid carcinoma of the ovary only

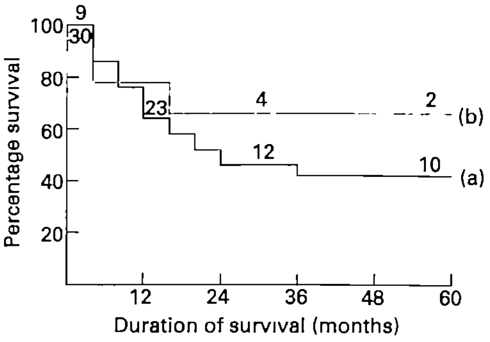

Fig. 2. Actuarial survival of women with endometrioid carcinoma of the ovary (a) and endometrioid carcinoma of the ovary and concomitant adenocarcinoma of the endometrium (b). (Numbers on graphs refer to the women at risk during that time interval.)

was not statistically significant. This finding may, in part, be due to the management of the women with concomitant tumours, the majority of whom presented with stage III ovarian disease and underwent optimal pelvic surgery, since successful surgery has been shown to be important with regard to patient survival (Einhorn *et al.* 1985). We have not been able to confirm that either the presence of a concomitant endometrial carcinoma or endometrial hyperplasia are prognostically favourable factors.

Over 750 women with endometrioid carcinoma of the ovary present each year in England and Wales (OPCS 1986) and approximately 150 of them will have a concomitant adenocarcinoma of the endometrium. A multicentred prospective trial would be of value in determining the full natural history, prognostic factors and optimal therapy for this neoplasm.

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Cost–benefit analysis of cephradine and mezlocillin prophylaxis for abdominal and vaginal hysterectomy

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Summary. Four hundred patients (300 abdominal and 100 vaginal hysterectomies) were randomized to receive a single, pre-operative intravenous injection of saline (placebo), 2 g cephradine or 5 g mezlocillin. The frequency of wound and pelvic infections was significantly reduced ($P<0{\cdot}05$, χ^2- or Fisher's exact test) in the abdominal hysterectomy patients who received cephradine (16% *vs* 23% mezlocillin, 29% placebo) and in the vaginal hysterectomy patients who received cephradine or mezlocillin (0% mezlocillin, 6% cephradine *vs* 27% placebo). These results are similar to those of previous studies and suggest that prophylaxis is more effective for vaginal than for abdominal hysterectomy. However, a cost–benefit analysis supported the opposite conclusion. Cephradine prophylaxis for abdominal hysterectomy resulted in cost savings to the hospital and the community health services with measurable benefits to the patient. In contrast, cephradine or mezlocillin prophylaxis for vaginal hysterectomy resulted in increased costs to the hospital, no savings to community services and no significant benefit to the patient. We conclude that cost–benefit analysis provided valuable additional information to the conventional, statistical analysis of wound or pelvic infection rates.

Placebo-controlled studies of antibiotic prophylaxis for hysterectomy have consistently shown a greater reduction in pelvic or wound infection rate for vaginal hysterectomy than for abdominal hysterectomy (Grossman *et al.* 1979; Polk *et al.* 1980; Savage *et al.* 1984; Sengupta *et al.* 1976; Stage *et al.* 1982), and the prophylaxis for abdominal hysterectomy remains controversial (Hirsch 1985). Although some previous studies have included estimates of cost savings to the hospital resulting from prophylaxis (Hemsell *et al.* 1983*a*; Polk *et al.* 1980; Sengupta *et al.* 1976) no previous study has attempted to quantify the impact of prophylaxis on community health services or on the patients' rate of recovery after discharge from hospital. This omission may be important; in one study 21% of wound infections and 31% of pelvic infections were first diagnosed after discharge from hospital (Polk *et al.* 1980).

In most previous studies, the prophylactic agent has been a first generation cephalosporin (Hirsch 1985). The commonest bacterial causes of wound and pelvic infection after abdominal and vaginal hysterectomy are coliforms and non-sporing anaerobic bacteria, principally species of *Bacteroides* and anaerobic cocci (Grossman *et*

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al. 1979; Polk *et al.* 1980; Savage *et al.* 1984; Sengupta *et al.* 1976; Stage *et al.* 1982).

The aims of the present study were to compare cephradine with mezlocillin, which has superior in-vitro activity against non-sporing anaerobic bacteria (Fass 1980), as prophylactic agents for abdominal or vaginal hysterectomy. The efficacy of prophylaxis was evaluated in a randomized, placebo-controlled double-blind trial. Cost–benefit analysis was used to assess the impact of prophylaxis on the patient, on the hospital and on community health services.

Patients and methods

The only patients excluded from the study were those who were allergic to penicillin and those who were considered to require a full course of therapeutic antibacterial treatment at the time of surgery. All other patients undergoing elective abdominal or vaginal hysterectomy were randomized to receive a single intravenous injection of saline (placebo), 2 g cephradine or 5 g mezlocillin at the time of induction of anaesthesia immediately before operation. A single randomization was used for abdominal and vaginal operations, patients were assigned to consecutively numbered boxes containing a single injection of one study drug which were packaged in unlabelled, numbered boxes by Bayer UK. A randomization code was held by the hospital pharmacy in case of adverse reaction. The injection was given by the anaesthetist; neither the study personnel nor the patient knew which agent had been given. Patients were anaesthetized with a combination of epidural and sleep anaesthesia. Verbal consent to participate in the study was obtained from all patients before surgery.

Assessment

All patients were assessed by a single observer (D.E.). The wound was inspected every third day in hospital and any evidence of infection at other sites was recorded. A wound was regarded as being infected if there was local inflammation. Pelvic infection was defined as a purulent vaginal discharge. Urinary infection was defined as a pure growth of $>10^5$ bacteria per ml of urine. Any therapeutic antibiotic therapy was recorded.

The severity of infection was not graded directly. However, two indirect indices were used: the percentage of patients whose infection was severe enough to require therapeutic antibiotics and the average cost of a course of therapeutic antibiotics.

All patients were contacted by letter and by telephone 2 weeks after discharge and asked to give details of any treatment which they had received at home for postoperative infection, including home visits from district nurses or family practitioners. The patient's recovery was assessed with the following scoring system:

Dusting, washing up: 1 point each; cooking, ironing, using stairs: 2 points each; making beds, changing beds, shopping, using a vacuum cleaner: 3 points each.

If necessary, patients were visited at home to confirm findings. All patients were reviewed in the outpatient department 6 weeks after discharge.

Costing

The price of all prophylactic and therapeutic antibiotics was calculated from the prices in the current MIMS. Only a minority of our patients receive antibiotics by the intravenous route. To test whether results were sensitive to a small number of patients receiving intravenous antibiotics, costs were also calculated using equivalent oral doses.

Statistics

Analysis of significance in fourfold tables was made with the χ^2-test with Yates' correction unless the total number of observations was <60 or the number in any cell was 0, when Fisher's exact test was used. Threefold or greater tables were analysed with the χ^2-test. Comparison of means was made with Student's *t*-test. The study duration was set at 2 years. Operation rates during the previous 5 years suggested that at least 300 patients would be recruited, which would be sufficient to demonstrate statistical significance between active and placebo groups based on infection rates in previously published studies. The study was unblinded on completion and the statistical analysis was performed by an independent analyst. None of the study or clinical personnel knew individual patient's randomization during the study.

The study was approved by the Ninewells Hospital ethics committee.

Table 1. Potentially important demographic variables in the three groups

	Abdominal hysterectomy			Vaginal hysterectomy		
	Cephradine (n = 97)	Mezlocillin (n = 101)	Placebo (n = 102)	Cephradine (n = 34)	Mezlocillin (n = 37)	Placebo (n = 29)
Age (years) Mean (SD)	44·2(9·53)	44·3(9·17)	44·0(8·78)	47·1(11·94)	46·5(10·86)	41·4(11·57)
Premenopausal	85(88)	88(87)	89(87)	23(68)	30(81)	24(83)
Benign pathology	85(88)	89(88)	92(90)	32(94)	32(86)	25(86)
Epidural anaesthetic	72(74)	73(72)	66(65)	24(71)	31(84)	17(59)
Operator						
Consultant	26(27)	30(31)	38(37)	10(30)	18(49)	4(14)
Senior registrar	33(34)	46(46)	33(32)	12(35)	17(46)	16(55)
Registrar	38(39)	24(24)	31(31)	12(35)	2(5)	8(28)
Abdominal preparation						
Chlorhexidine	87(90)	81(80)	96(94)	NA	NA	NA
Clorhexidine plus povidone iodine	10(10)	20(20)	6(6)			
Vaginal preparation						
Gentian violet	78(80)	88(88)	91(89)	0	0	0
Povidone iodine	19(20)	12(12)	11(11)	0	0	0
Cetrimide	0	0	0	34(100)	37(100)	29(100)
Abdominal closure						
Suture	85(88)	84(83)	93(91)	NA	NA	NA
Clips	12(12)	17(17)	9(9)			
Duration of operation						
<1 h	42(43)	60(59)	57(56)	22(65)	27(73)	20(69)
1–2 h	53(55)	38(38)	45(44)	12(35)	10(27)	9(31)
>2 h	2(2)	3(3)	0	0	0	0

Results are numbers of patients with the rate in the group as % in brackets. NA, Not applicable.

Results

A total of 420 patients entered the study but 20 were excluded because the consultant decided that the patients required a full therapeutic course of antibiotics at the time of surgery. Of the 400 remaining patients, 300 had abdominal hysterectomies and 100 had vaginal hysterectomies. In general the patients were well randomized between the three treatment groups for each operation (Table 1). Epidural anaesthesia was the only variable which differed significantly between the three groups; fewer patients in the placebo group had epidural anaesthesia for either adominal or vaginal hysterectomy. However, the only variables in Table 1 which were significantly associated with postoperative infection were malignant or premalignant pathology and duration of operation.

The frequency of postoperative infection was significantly higher in patients with underlying malignant disease (47% with malignant diseases compared with 26% with no malignant disease, χ^2 7·24, P<0·01). However, the number of patients with benign pathology was similar in each group (Table 1). The risk of wound and pelvic infection increased with duration of operation in the placebo and cephradine groups but was consistently lower in the cephradine group than in the placebo group (Fig. 1). In contrast, wound and pelvic infection rates in the mezlocillin group were only lower than in the placebo group for operations which lasted >1 h (Fig. 1).

Cephradine prophylaxis reduced the frequency of pelvic/wound infections and urinary infections observed in hospital after hysterectomy, whereas mezlocillin only reduced the frequency of urinary infection (Table 2). In the vaginal hysterectomy patients both cephradine and mezlocillin reduced the frequency of pelvic infection observed in hospital but neither drug reduced the frequency of urinary infection (Table 2).

Of the 79 pelvic or wound infections which were observed, 27 (34%) occurred at home, after discharge from the hospital; after inclusion of these infections the frequency in the

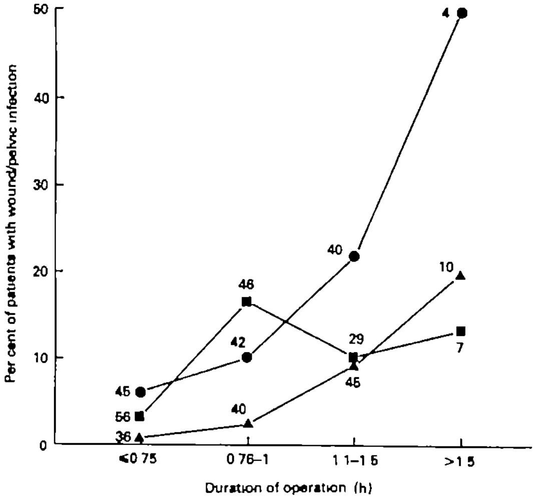

Fig. 1. Rates of wound/pelvic infection after hysterectomy by duration of operation in three groups treated with prophylactic cephradine (▲), mezlocillin (■) or placebo (●). (Numbers indicate the number of women.)

cephradine group of abdominal hysterectomy patients (16/97) was not significantly lower than that in the placebo group (29/102) (χ^2 3·39).

In comparison with the placebo group, significantly fewer patients received therapeutic antibiotics postoperatively in the cephradine and mezlocillin groups after abdominal hysterectomy and in the mezlocillin group after vaginal hysterectomy (Table 3). The proportion of patients with any infection diagnosed in hospital who received antibiotics was significantly lower in the cephradine and mezlocillin groups after abdominal hysterectomy (placebo 39/42, cephradine 9/16, χ^2 8·46, *P*<0·01; mezlocillin 16/30, χ^2 13·05, *P*<0·001). Antibiotic prescription was used as the index of infection diagnosed by general practitioners, therefore all of these patients received antibiotics. In addition, the average cost of a course of antibiotics tended to be higher in the placebo group than in either of the prophylaxis groups for vaginal or abdominal hysterectomy (Table 4). Because of small numbers and large standard deviations, these differences were statistically significant only when the cost of all antibiotics for abdominal hysterectomy were combined (placebo mean £9.23, cephradine mean £6.19, *t* 2·526, *P*<0·02; mezlocillin mean £6.25, *t* 2·41, *P*<0·02). There was no difference in the types of therapeutic antibiotic between the three groups. Only four patients received intravenous antibiotics (three in the placebo group). Because of the greater cost of intravenous antibiotics, the analysis in Table 4 was done with the cost of equivalent oral doses as well as for the drugs actually prescribed. More patients in the cephradine group received

Table 2. Infections diagnosed in hospital and by general practitioners. Some patients had more than one infection so that the total with any infection may be less than the sum of individual infections

	Abdominal hysterectomy			Vaginal hysterectomy		
	Cephradine (n = 97)	Mezlocillin (n = 101)	Placebo (n = 102)	Cephradine (n = 34)	Mezlocillin (n = 37)	Placebo (n = 29)
Infections diagnosed in hospital						
Wound/pelvic	6*	18	20	1†	0††	6
Urinary tract	10*	10*	21	7	6	3
Chest	2	4	8	0	0	1
Total with any infection diagnosed in hospital	16**	30	42	8	6	10
Infections diagnosed by general practitioner						
Wound/pelvic	10	4	9	1	0	2
Urinary tract	12	8	4	8	2	5
Chest	4	3	2	1	0	0
Total with any infection diagnosed at home	25	14	15	10	2	7
Total with any infection, in hospital or at home	40	40	53	14	7*	15

Significance of differences compared with placebo * *P*<0·05, ** *P*<0·01 (χ^2-test with Yates' correction); † P<0·05, ††*P*<0·01 (Fisher's exact test).

Table 3. Number (%) of patients who received therapeutic antibiotics for postoperative infection

	Cephradine	Mezlocillin	Placebo
Abdominal hysterectomy			
Hospital only	9(10)	16(16)	39(38)
General practitioner (GP) only	25(26)	14(14)	15(15)
Hospital and GP	5(5)	5(5)	3(3)
Same infection	4(4)	4(4)	2(2)
Different infection	1(1)	1(1)	1(1)
Total	39(40)	35(35)	57(56)
χ^2 *vs* placebo	4·3	8·4	
P	<0·05	<0·01	
Vaginal hysterectomy			
Hospital only	4(12)	5(14)	7(24)
General practitioner (GP) only	10(29)	2(5)	7(24)
Hospital and GP	0	1(3)	0
Same infection		1(3)	
Different infection		0	
Total	14(41)	8(22)	14(48)
χ^2 *vs* placebo	1·3	6·36	
P	>0·1	<0·02	

antibiotics from general practitioners after abdominal hysterectomy (25 *vs* 15 placebo). However, the total cost of GP antibiotics was similar (£148.16 cephradine *vs* £159.54 placebo) because the average cost per course was higher in the placebo group.

The distribution of recovery scores for the abdominal hysterectomy patients was signifi-

Table 4. Total and average costs per patient (£) for antibiotics prescribed by the hospital and by general practitioners for treatment of postoperative infection*

	Cephradine	Mezlocillin	Placebo
Abdominal hysterectomy			
Hospital antibiotics			
Intravenous and oral			
Total	93.36	142.97	539.56
Mean (SD) per patient	6.66 (7.81)	6.81 (5.26)	12.85 (16.11)
Oral only			
Total	93.36	142.97	404.62
Mean (SD) per patient	6.66 (7.81)	6.81 (5.26)	9.63 (6.20)
General practitioner antibiotics			
Total	148.16	75.88	159.54
Mean (SD) per patient	4.94 (5.69)	3.99 (2.65)	8.86 (6.60)
Vaginal hysterectomy			
Hospital antibiotics			
Intravenous and oral			
Total	125.25	22.00	167.12
Mean (SD) per patient	31.3 (53.3)	3.67 (1.18)	23.87 (25.78)
Oral only			
Total	20.00	22.00	84.54
Mean (SD) per patient	5.00 (1.78)	3.67 (1.18)	12.08 (3.60)
General practitioner antibiotics			
Total	43.04	22.20	36.78
Mean (SD) per patient	4.30 (1.87)	7.40 (6.44)	5.25 (3.60)

* Fourteen patients received prescriptions from both sources. The appropriate costs were allocated to the hospital and general practitioner totals

Table 5. Recovery scores after vaginal or abdominal hysterectomy, number (%) in each category

	Abdominal hysterectomy			Vaginal hysterectomy		
	Cephradine	Mezlocillin	Placebo	Cephradine	Mezlocillin	Placebo
Recovery score						
0–4	8(9)	24(24)	23(23)	2(6)	2(6)	5(17)
5–9	30(30)	16(16)	25(24)	5(15)	6(16)	8(28)
10–20	59(61)	61(60)	54(53)	27(79)	29(78)	16(55)
χ^2_2 *vs* placebo	6·52	2·42	4·4	4·4		
P	<0·05	>0·1		>0·1	>0·1	

Higher scores indicate better recovery (see Patients and methods).

cantly different in the placebo and cephradine groups (Table 5). The difference was accounted for by a greater proportion of patients with scores of 0–4 (no work or light work only) in the placebo group. There were no significant differences in recovery scores amongst the vaginal hysterectomy patients, although the distribution in the placebo group tended towards lower scores than in the cephradine or mezlocillin groups (Table 5).

The total cost of prophylactic and therapeutic antibiotics for the abdominal hysterectomy patients was lowest in the cephradine group and highest in the mezlocillin group (Table 6). Even after exclusion of intravenous antibiotics, total antibiotic costs were similar in the cephradine and placebo groups (£590.72 cephradine *vs* £504.08 placebo). There were additional potential savings to the Health Service from prophylaxis for abdominal hysterectomy. Patients in the cephradine and mezlocillin groups had shorter hospital stays and received fewer home visits from family practitioners or district nurses than patients in the placebo group (Table 6). The reduction in district nurse visits was a product of a lower number of patients visited (8 cephradine, 11 mezlocillin, 16 placebo) and a lower number of visits per patient visited (6·9 cephradine, 5·6 mezlocillin, 12·6 placebo). In contrast, for the vaginal hysterectomy patients the placebo group had the lowest total antibiotic costs and there was no consistent evidence of additional savings from prophylaxis. None of the patients required home visits from district nurses and, although the number of family practitioner visits was lowest in the cephradine group, this group had the longest average stay in hospital (Table 6).

Discussion

Hirsch (1985) has reviewed all placebo-controlled trials of antibiotic prophylaxis for abdominal

Table 6. Total antibiotic costs and other potential costs to the health service arising from postoperative infection. The hospital antibiotic costs are for intravenous and oral antibiotics

	Vaginal hysterectomy			Abdominal hysterectomy		
	Cephradine	Mezlocillin	Placebo	Cephradine	Mezlocillin	Placebo
Antibiotic costs (£)						
Prophylactic	132.60	282.31	0	349.20	770 63	0
Therapeutic:						
Hospital	125.25	22.00	167 12	93.36	142 97	539.56
General practitioner	43.04	22.20	36.78	148 16	75 88	159 54
Total	300.89	326.51	203.90	590 72	985.57	699.10
Other potential costs						
Days in hospital (*n*)						
Mean	8·1	7·3	7·2	8·0	7·9	8·7
95% CI	7·2–9·0	7·0–7·6	6·7–7·7	7·7–8·3	7·6–8·2	8·2–9·2
Family practitioner visits (*n*)	11	17	16	58	54	82
District nurse visits (*n*)	0	0	0	55	62	195

and vaginal hysterectomy published up to December 1983. Antibiotic prophylaxis reduced the frequency of pelvic infection in 5524 vaginal hysterectomy patients from 25 to 5%, whereas in 3752 abdominal hysterectomy patients prophylaxis only reduced the rate of wound and pelvic infection from 18 to 10%. These results are very similar to those obtained with cephradine in the present study (Table 2). Hirsch (1985) did not give figures for urinary tract infections but Polk *et al.* (1980) reported similar results to the present study in that cefazolin prophylaxis caused a significant reduction in urinary tract infection after abdominal hysterectomy but not after vaginal hysterectomy. These results may be attributable to a longer duration of urinary catheterization after vaginal hysterectomy (Polk *et al.* 1980).

Analysis of crude pelvic and wound infection rates from the present study supports the conclusion (Hirsch 1985) that prophylaxis is more beneficial for vaginal hysterectomy than for abdominal hysterectomy. The results of the cost–benefit analysis were quite different. Total antibiotic costs for abdominal hysterectomy patients were lower in the cephradine group than in the placebo group because a smaller proportion of patients with infection received antibiotics and because the average cost per course was lower, suggesting that prophylaxis reduced the severity of infection as well as the occurrence of infection. Cephradine prophylaxis resulted in additional potential savings to the Health Service (Table 6) as well as faster recovery by the patients (Table 5). There was no evidence that the savings to the hospital were made at the expense of increased cost to the community. The cost of antibiotics prescribed by general practitioners was similar in the cephradine and placebo groups (Table 4) and the cephradine patients received fewer district nurse and general practitioner visits (Table 6). In contrast, total antibiotic costs for vaginal hysterectomy patients were lowest in the placebo group, there were no additional benefits to the Health Service (Table 6) from prophylaxis and only a non-significant trend towards faster recovery (Table 5). Although the unequal numbers of abdominal and vaginal hysterectomy patients preclude direct comparison, cost–benefit analysis (Drummond *et al.* 1987) supports cephradine prophylaxis for abdominal hysterectomy, whereas the inconsistent benefits of cephradine or mezlocillin prophylaxis for vaginal hysterectomy must be weighed against the added cost. Morbidity from pelvic infection after vaginal hysterectomy should not be assumed to be equivalent to morbidity from pelvic or wound infection after abdominal hysterectomy.

In the present study cephradine prophylaxis was effective in preventing wound or pelvic infection regardless of the duration of operation. However, only 5% of operations lasted >1·5 h, whereas, in a study by Shapiro *et al.* (1982) 66% of operations lasted >1·75 h and cefazolin prophylaxis did not prevent infections after abdominal hysterectomies which lasted >3·5 h. Our results may not be relevant to centres with longer operation times.

Despite its superior in-vitro activity against coliforms and non-sporing anaerobic bacteria, mezlocillin was less effective than cephradine as prophylaxis for abdominal hysterectomy and only marginally more effective for vaginal hysterectomy (Table 2). In earlier studies cefazolin (Hemsell *et al.* 1983*b*) or cefoperazone (Hemsell *et al.* 1984) were as effective as cefoxitin (Hemsell *et al.* 1984) or moxalactam (Hemsell *et al.* 1983*b*) as prophylaxis for abdominal hysterectomy, although resistance to cefoxitin and moxalactam is less common amongst coliforms and non-sporing anaerobic bacteria than resistance to cefazolin and cefoperazone (Anonymous 1982). Moreover, Grossman *et al.* (1979) showed that benzyl penicillin was as effective as cefazolin in preventing infection after abdominal or vaginal hysterectomy. Further work is required on the relative efficacy of prophylactic antibacterial drugs but the available evidence does not support the theory that in-vitro activity against coliforms and non-sporing anaerobic bacteria is a good guide to the selection of prophylactic drugs for abdominal hysterectomy.

In conclusion, a simple cost–benefit analysis provided valuable additional information to the conventional, statistical analysis of pelvic and wound infection rates after hysterectomy. Methods for economic appraisal merit wider application in clinical trials (Drummond *et al.* 1987; Leu 1985).

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Bladder drainage: a survey of practices among gynaecologists in the British Isles

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Summary. All 2836 members and fellows of the Royal College of Obstetricians and Gynaecologists were circulated with a questionnaire concerning catheterization practices; 1229 replies were received, an overall response rate of 43%; the response rate from practitioners of consultant and senior registrar status was 67%. Practices varied considerably, some aspects of management apparently relating to the age or experience of the surgeon, some to their level of interest or surgical commitment in gynaecological urology, and some showing regional variation. Of the 960 respondents in active gynaecological practice, 84–93% (depending on the operation) drained the bladder before routine abdominal procedures, 52–54% (depending on the route) drained the bladder before incontinence surgery, and 62–70% routinely used continuous bladder drainage for periods between 1 and 12 days following these procedures. Overall, 51% of respondents preferred urethral, and 39% suprapubic catheters for postoperative bladder drainage; this showed a marked regional- and experience-related variation. Prophylactic antibiotics were used by 32% of gynaecologists overall, and a wide variety of other measures were employed in the management of catheter-associated infection, and other problems of catheter management.

Catheterization of the bladder is one of the most commonly performed surgical procedures, used in up to 20% of all patients in hospital (Stevens *et al.* 1981). It is the commonest cause of infection acquired in hospital, accounting for approximately 35% of such events in the USA (National Nosocomial Infection Study 1973) and UK (Report on the National Survey of Infection in Hospitals 1981). Catheter-associated infection is estimated to occur in 10^6 of the 40×10^6 individuals hospitalized annually in the USA (Thompson *et al.* 1984), and of these approximately 1% will become bacteraemic with a threefold increase in mortality (Platt *et al.* 1982). For the remainder catheterization may mean considerable discomfort, longer stay in hospital, and major financial implications for health services (Givens & Wenzel 1980).

Despite these major implications, it is perhaps the very familiarity which both medical and nursing staff feel they have with catheterization which leads them all too often to neglect the basic principles of bladder drainage. Whilst much has been written about the techniques and complications of catheterization, reviewed by Hilton (1987), there is little information concerning actual practices. The aim of this study was to establish current practices with respect to bladder drainage among gynaecologists in the British Isles.

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Methods

All members and fellows registered with the Royal College of Obstetricians and Gynaecologists (RCOG) at the end of 1985 and resident within the British Isles were circulated with a reply-paid questionnaire relating to catheter practices. Most of the questions were of a closed nature requiring respondents to tick one of a series of mutually exclusive answers. The questionnaire included: the use of bladder drainage before, during and after gynaecological and obstetric surgery; preference for suprapubic or urethral catheterization; type of suprapubic catheter preferred; characteristics of urethral catheters preferred for short- and long-term drainage; the use of prophylactic measures against catheter-associated infection; and approaches to other complications of catheter management. In an effort to assess regional variations in practice and the influence of experience and specialist interests within gynaecology, respondents were also asked to state the time since they had obtained membership (MRCOG), the region in which they were currently employed, whether they had a major specialist interest in gynaecological oncology or urology, and the extent of their surgical workload in general gynaecology or in these specialist areas.

Results

Respondents

A total of 2836 questionnaires was distributed, and 1229 were returned, giving an overall response rate of 43%. Of the returns, 269 were uncompleted: 153 from retired members or fellows, 84 from members practising in specialities other than obstetrics and gynaecology, 29 were returned by the Post Office undelivered, and three were returned by relatives of deceased members or fellows; 960 completed questionnaires were returned by members and fellows currently active within the speciality—'the appropriate respondents'—(78% of returns; 34% of the total circulation). Of the 960 appropriate respondents 688 (72%) were of consultant status, 133 (14%) of senior registrar status, and 118 (12%) were registrars; the remaining 21 (2%) were made up of hospital practitioners, clinical assistants, senior house officers or of unspecified status. Based on predicted figures for 1986 from the Report of the Manpower Advisory Sub-Committee of the RCOG (1983) the response rate from consultants was approximately 65% and from senior registrars 78%.

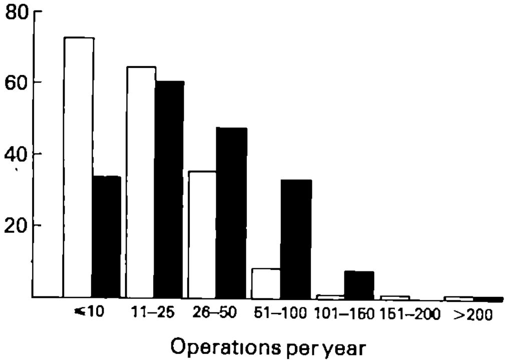

Fig. 1. Annual incontinence surgery workload of the 186 respondents with a major specialist interest in gynaecological urology. □, Suprapubic incontinence operation; ■, vaginal incontinence operation

Of the 960 appropriate respondents, 186 (19%) considered themselves to have a major specialist interest in gynaecological urology; Fig. 1 shows that their surgical workload varied considerably. In several areas of the subsequent analysis the practices of the 774 surgeons with no particular interest in gynaecological urology were compared with the 186 who claimed a major specialist interest and the 48 surgeons who had a significant surgical workload in the subspeciality, arbitrarily defined for these purposes as more than 25 suprapubic incontinence procedures per year.

There were 180 (19%) appropriate respondents who considered themselves to have a major specialist interest in gynaecological oncology and 58 of them had a significant surgical workload in the subspeciality, defined for these purposes as more than 25 radical cancer procedures per year. Again, the practices of the 780 surgeons who had no particular interest in gynaecological oncology were compared with the practices of these two subgroups.

Altogether 302 respondents (31%) considered themselves to have a major specialist interest in either gynaecological oncology or urology; 64 respondents (7%) claimed to have a major specialist interest in both of these areas.

The use of routine catheterization during operation

Respondents were asked to state whether they would routinely empty the bladder before operation, would leave an in-dwelling catheter on free drainage during the operation, and would use continuous bladder drainage as a routine postoperatively, for a range of obstetric and gynaecological procedures; where postoperative drainage was advocated they were asked to specify the duration of free drainage. Responses are shown in Table 1. At standard abdominal operations, between 84 and 93% of respondents (depending on procedure) routinely drained the bladder before operation. Between 6 and 8% used prophylactic continuous bladder drainage postoperatively, although greater use was made of catheterization following caesarean section under epidural anaesthesia, where 27% drained the bladder electively.

Table 1. The use of routine bladder drainage before, during, and after gynaecological and obstetric surgery

	Percentage of respondents using catheterization routinely at surgery			Duration of post-operative drainage (days)	
Surgical procedure	Before	During	After	Median	(Range)
Caesarean section					
General anaesthesia (*n* = 959)	85	44	8	0	(0–2)
Epidural anaesthesia (*n* = 955)	84	56	27	0	(0–7)
Abdominal hysterectomy (*n* = 959)	93	14	7	0	(0–7)
Other laparotomy (*n* = 959)	87	9	6	0	(0–5)
Vaginal hysterectomy (*n* = 959)	62	10	35	0	(0–7)
Anterior colporrhaphy (*n* = 959)	54	14	70	2	(0–7)
Suprapubic incontinence procedure (*n* = 920)	52	36	62	3	(0–12)
Radical hysterectomy (*n* = 890)	77	46	53	2	(0–28)

n, Numbers of respondents completing item and therefore presumed to perform procedure.

Table 2. The effect of surgeon's level of interest in gynaecological urology or oncology on the use of routine catheterization at anterior colporrhaphy and suprapubic incontinence surgery, or radical hysterectomy respectively

	Percentage of respondents using catheterization routinely at surgery			Duration of post-operative drainage (days)	
Surgical procedure	Before	During	After	Median	(Range)
Anterior colporrhaphy					
Generalist surgeons (*n* = 773)	54	12	71	2	0–7
Major urology interest (*n* = 186)	55	19	* 67	2	† 0–7
Major interest with >25 specialist operations/year (*n* = 48)	47	16	84 *	3	0–5 †
Suprapubic incontinence operation					
Generalist surgeons (*n* = 734)	51	34	57	3	0–12
Major urology interest (*n* = 186)	56	42	** 80 **	4	0–12
Major interest with >25 specialist operations/year (*n* = 48)	41	31	80	4	0–8
Radical hysterectomy					
Generalist surgeons (*n* = 712)	78	43	48	2	0–28
Major oncology interest (*n* = 178)	75	56	** 73 **	5	†† 0–28 ††
Major interest with >25 radical operations/year (*n* = 58)	76	59	79	7	0–28

Significance of difference * $P<0{\cdot}05$, ** $P<0{\cdot}001$ (χ^2-test); † $P<0{\cdot}05$, †† $P<0{\cdot}001$ (Mann–Whitney *U*-test).
n, Numbers of responses, the disparities in numbers reflect different response rates to various questions.

At prolapse or incontinence surgery 52 to 62% drained the bladder before operation, depending on procedure. Following anterior colporrhaphy 70% drained the bladder continuously for periods between 1 and 7 days (mean 2·1 days); following suprapubic incontinence surgery 62% drained continuously for between 1 and 12 days (mean 2·6 days). The surgeon's interest in urology had little influence on the practice of catheterization before operation, but did affect postoperative bladder management (see Table 2). Those surgeons with a major specialist interest in gynaecological urology and a significant incontinence surgery workload made more frequent use of postoperative bladder drainage, 84% catheterizing prophylactically following anterior colporrhaphy, and 80% following suprapubic incontinence procedures; both proportions are statistically significantly higher than those for generalist surgeons, 71% and 57% respectively. The duration of free bladder drainage following anterior colporraphy (but not suprapubic procedures) was significantly longer for those respondents with a significant incontinence surgery workload compared to the other groups.

At radical hysterectomy (Table 1) 77% drained the bladder before operation, and 53% drained postoperatively for periods between 1 and 28 days (mean 3·0 days). Specialist interest in oncology made no difference to the practice of pre-operative catheterization, but did affect the use of postoperative bladder drainage (Table 2). Of generalist surgeons, 48% drained the bladder postoperatively, whereas, of those professing a special interest in oncology and with a significant oncological surgical workload, 79% employed routine postoperative catheterization; the duration of postoperative bladder drainage was also significantly different, those with the greater commitment to gynaecological oncology catheterizing for longer periods.

Preferred route of bladder drainage for postoperative use

Respondents were asked to state their preference for urethral or suprapubic catheterization for routine postoperative bladder drainage. Overall, 494 (51%) preferred the urethral route, 372 (39%) the suprapubic route, and 94 (10%) had no preference. Of the generalist surgeons 36% preferred suprapubic catheters, a significantly lower percentage than among those professing a special interest in urology, and those with a significant incontinence surgery workload, of whom 51% and 63% respectively advocated this route ($P<0{\cdot}001$, χ^2-test).

There was a consistent negative trend in the preference for suprapubic catheterization with time since obtaining membership of the RCOG, 50% of those with ≤10 years of membership preferred this route compared with 20% of those with >40 years of membership (Fig. 2).

Regional differences in the preference for suprapubic catheterization were also marked, with greater advocacy of this route among the gynaecologists in the health regions of the south of England (Fig. 3a). Overall preference figures varied from 17% in the North Western region to 62% in the Oxford region. This regional distribution was seen in gynaecologists of all ages, although it was more marked in those with >10 years membership, varying from 10% in the North Western region to 69% in the Oxford region (Fig. 3b); gynaecologists with ≤10 years membership were somewhat more consistent in their preferences with between 26% and 64% advocating the suprapubic route.

Suprapubic catheter types

The 574 respondents who at any time used suprapubic catheters were asked to state which

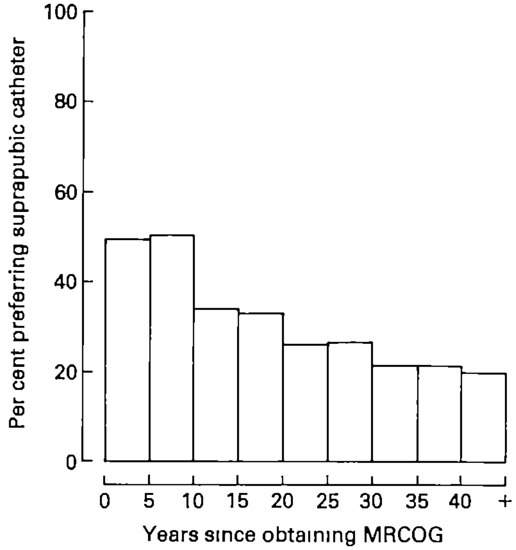

Fig. 2. Trends in preference for suprapubic catheterization with time from obtaining membership of the Royal College of Obstetricians and Gynaecologists (MRCOG).

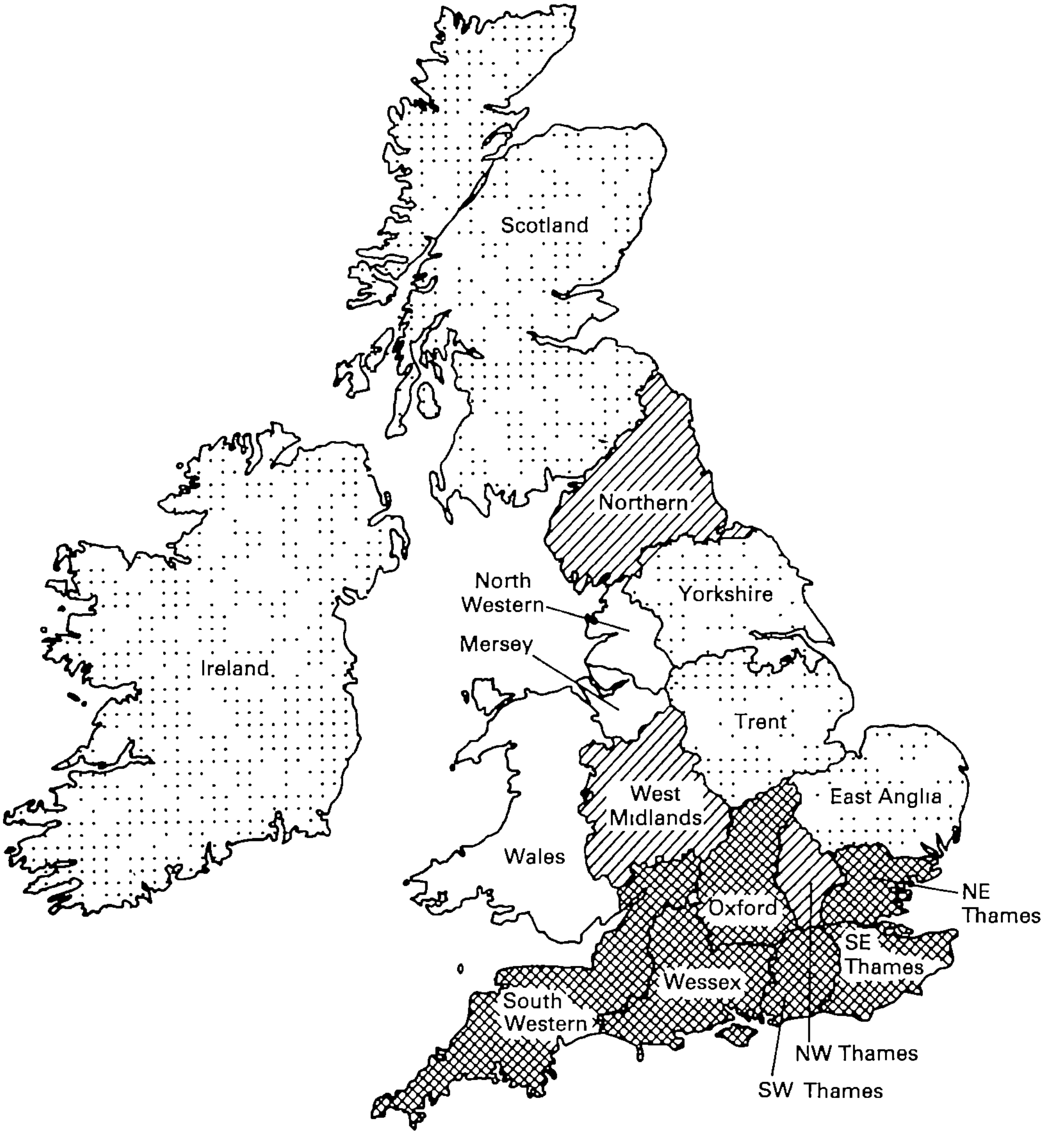

Fig. 3a. Regional variations in suprapubic catheter use—all respondents. □, ≤25%; ⊡, 26–33%; ▨, 34–50%; ▩, 51–66%; ■, ≥67%.

device they used most often. Most (98%) used a pre-packed system designed for closed suprapubic stab insertion: 413 (72%) used the Becton-Dickinson 'Bonanno' catheter, 104 (18%) the Dow-Corning 'Cystocath', 20 (3%) the Argyle 'Ingram' catheter, 15 (3%) the Franklin 'Simplastic' catheter, 5 (1%) the Braun 'Cystofix', 4 (1%) the Cook 'Stamey' catheter; almost all the remainder (11/13) used a Foley catheter presumably inserted at open cystotomy or by the technique of cutting down onto a sound. There were no regional variations in the preferred suprapubic catheter type, and no significant differences related to special interest in urology.

Urethral catheter types

Respondents were asked to indicate on several lists the characteristics that they would select in a urethral catheter for postoperative and long-term bladder drainage (Table 3). The majority

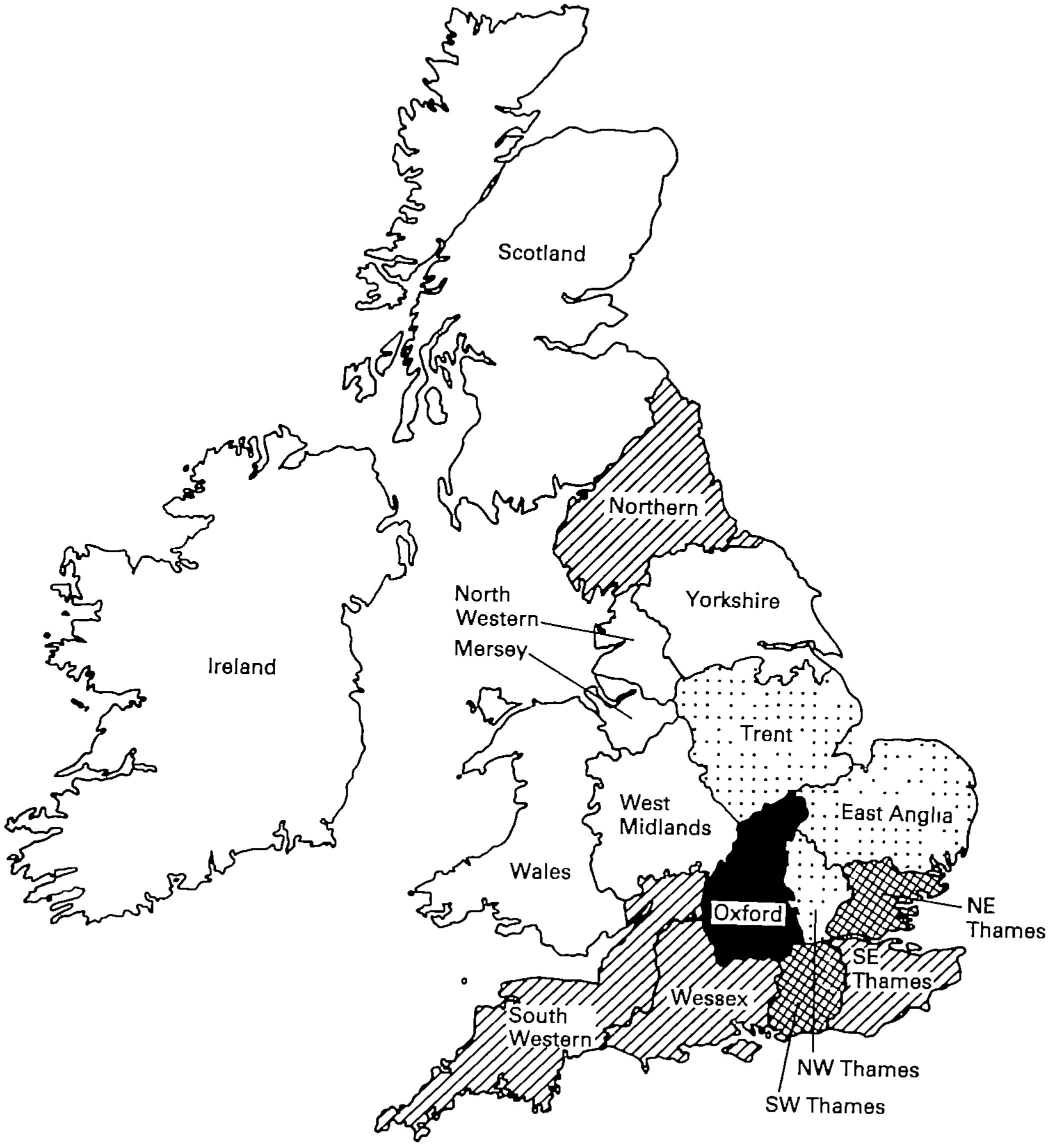

Fig. 3b. Regional variations in suprapubic catheter use—respondents with >10 years membership of the RCOG For key see legend to Fig 3a.

(approximately two-thirds in each instance) advocated simple latex catheters for post-operative use, and all-silicone catheters for long-term drainage. A similar proportion advocated the use of 14–16 French gauge catheters and 5–10 ml retaining balloons, and approximately one-third use 10–12 gauge catheters for both short- and long-term drainage. Only one in four respondents used a female length catheter in any situation and 310 (32%) relied entirely on nursing staff to select catheters. None of these data was significantly affected by the surgeon's level of interest in urology.

Prophylaxis against catheter-associated infection

Respondents were asked whether they currently employed prophylactic antibiotic cover or several other measures to prevent infection in catheterized patients on a routine basis (Table 4). Practices were again unaffected by a sur-

Table 3. Characteristics of urethral catheters selected for postoperative and long-term bladder drainage

Characteristics	Percentage of gynaecologists selecting for: Postoperative use	Long-term use
Material		
Latex	64	17
Coated latex	25	15
100% silicone	9	68
Other	1	0
Calibre		
10–12 FG	36	31
14–16 FG	62	67
18–20 FG	2	2
Other	0	0
Balloon size (ml)		
3–5	16	16
5–10	66	61
30–50	6	10
15 ml in 30 ml balloon	12	12
Double balloon	0	0
Length		
Standard	76	
Female	24	

Table 4. The routine use of prophylactic measures against catheter-associated infection

	n	(%)
Prophylactic antibiotics	314	(33%)
Perineal or meatal toilet	286	(30%)
Antiseptic in drainage bag	59	(6%)
Bladder irrigation	45	(5%)
High volume output	452	(47%)
Other	9	(1%)
Noxythiolin washout	3	
Chlorhexidine washout	1	
Acidification of urine	3	
Vaginal antiseptic cream	1	
Chlorhexidine bidet	1	

geon's level of interest in urology, although the use of prophylactic antibiotics did vary with experience, increasing from 23% of gynaecologists with ≤5 years membership to 61% of those with >40 years membership (Fig. 2). The use of bladder irrigation showed a similar trend, increasing from 4% to 9% for the same groups

Problems of catheter management

Respondents were asked to state their usual approach to the following problems of catheter management:

Leakage around a catheter. Of 851 gynaecologists who responded to this question over 80% advocated removal or replacement of the catheter, and over 50% suggested replacement with a similar size or larger catheter; fewer than 5% suggested a smaller catheter or retaining balloon (Table 5).

Persistent voiding difficulty. A wide variety of responses was obtained to this problem (Table 6). Half the respondents mentioned recatheterization, and of those stating duration the modal time was 2 days. Various pharmacotherapies were used by 29% and whilst most of the drugs mentioned were theoretically appropriate, 10% were potentially detrimental to voiding efficiency (anti-spasmodics, diuretics and mazindol).

Intermittent clamp-release regimens

Respondents were asked whether they ever used intermittent clamping and release regimens in patients with urethral catheters; 528 (55%) responded positively. The technique was used most frequently by 'generalist gynaecologists' (57%), and significantly less often by those with a major interest in gynaecological urology (48%), and those with a significant incontinence surgery workload (39%) ($P<0{\cdot}05$ and 0·02, respectively, χ^2-test).

Clean intermittent self-catheterization

Respondents were asked to state whether they saw any place in their own practice for the technique of clean intermittent self-catheterization, and if so, then how many patients they currently had undergoing this form of management. Overall, 237 (25%) saw a place for self-catheterization and approximately 365 patients were currently using the technique under the care of 102 gynaecologists. Of these, over half have only one patient currently undergoing this form of management, and only nine have 10 or more patients treated in this way.

Of the 186 gynaecologists with a major specialist interest in urology, only 63 (34%) find a place for clean intermittent self-catheterization; 261 patients are currently undergoing this form of management under the care of 36 such gynaecologists who each handle on average

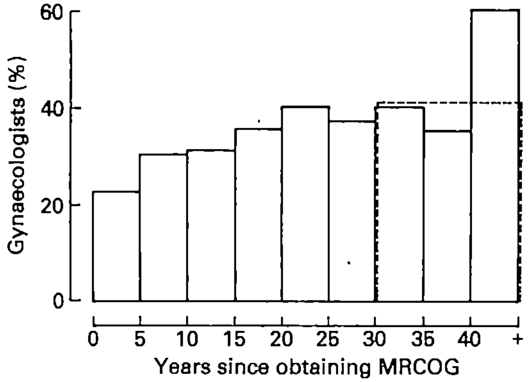

Fig. 4. Trends in the use of prophylactic antibiotics in catheterized patients with time from obtaining MRCOG (dotted column represents mean for those with >30 years membership).

seven such patients. Conversely, 104 patients are currently undergoing this form of management under the care of 66 gynaecologists with no special interest in urology who each deal with only one or two such patients.

Discussion

Reaching firm conclusions from a survey with a response rate of 43% is, of course, difficult, since responders may be systematically different from non-responders, particularly in their level of interest in the problem under investigation. Notwithstanding this criticism, the most appropriate group to survey are perhaps those of consultant and senior registrar status, whose response rate averaged 67%. The respondents in this study show a broad spectrum of age, subspeciality interests, and regional distribution. There were over 100 respondents in each 5-year group from 0–5 years to 21–25 years of membership; one in five respondents professed a major specialist interest in gynaecological urology, a similar number an interest in gynaecological oncology. Exact information does not exist about the distribution of gynaecologists by employing health region; however, by reference to the RCOG territorial lists and the Report of the Manpower Advisory Sub-Committee (1983), it would seem that the response rates varied between approximately 60 and 100% from consultants and senior registrars, for the different regions considered.

One might expect that the differences in practice between those surgeons professing a major specialist interest within gynaecology, and those without such an interest, would perhaps reflect their training and experience of that subject, and that their practices might therefore lead the way to improved standards. In terms of postoperative catheterization their views would point towards more widespread use of catheterization following prolapse or incontinence surgery, and radical hysterectomy, with drainage for longer periods than is employed by 'generalist' gynaecologists.

Those with an interest in urology more frequently advocate the suprapubic route for catheterization. The regional variation with regard to the use of suprapubic catheters is intriguing and on the data collected does appear to show a genuine North–South gradient. This does not reflect a greater interest in urology among gynaecologists in the south of England; although the proportion claiming a major specialist interest in urology does vary between regions, from 11% to 31%, there is no consistent national gradient. The use of suprapubic catheters was also shown to have a marked age-related variation; again there was a regional variation in age of respondents, but not showing any consistent North–South gradient. There is an age-related variation in the proportion of gynaecologists with a urological interest—14% of respondents with ≤10 years of membership professing a major specialist interest in urology, compared with 22% of those with 11–20 years, 23% of those with 21–30 years, and 32% of those with ≥31 years of membership. These three variables would seem to be quite independent, the preference for suprapubic catheters being greater in the south of England, in more recently qualified gynaecologists, and in those with an interest in urology. To examine the extremes, of those from the North Western region with >10 years of membership and with no special interest in urology, only 3 of 39 (8%) prefer suprapubic catheters, whereas of those younger respondents from the Oxford or Thames regions with an interest in urology, 13 of 16 (81%) advocate this route.

Several studies have investigated the use of suprapubic catheterization in gynaecological practice. Following pelvic surgery, particularly for the treatment of urinary incontinence, suprapubic catheterization has the advantage that the patient's ability to void can be tested without removing the catheter; this has been shown to appreciably reduce patient discomfort, nursing time, and urinary infection, since repeated catheterizations are unnecessary (Bonanno *et al.* 1970; Mattingly *et al.* 1972). It

Table 5. Methods applied in the management of urine leakage around a catheter

Method	Respondents (*n* = 851)	
	n	(%)
Replace catheter	566	(66%)
With same	232	(27%)
Increase calibre	246	(29%)
Increase balloon size	35	(4%)
Increase or decrease calibre or balloon	22	(3%)
Decrease balloon size	19	(2%)
Suprapubic catheter	12	(1%)
Remove catheter	143	(17%)
Check for blockage	113	(13%)
Anti-spasmodic drugs	20	(2%)
Check for fistula	5	(1%)
Bladder washout	3	(0%)
Phenazopyridine	1	(0%)

Table 6. Methods of management applied to the problem of persistent voiding difficulty after catheter removal (urethral) or clamping (suprapubic)

Management	*n*	(%)
Modification of catheter regimen		
Wait	76	(8%)
Recatheterize	396	(41%)
Duration specified	92	
1 day	9	
2 days	65	
3 days	8	
5 days	6	
7 days	3	
2–4 weeks	1	
Replace urethral catheter by suprapubic	50	(5%)
Replace suprapubic catheter by urethral	4	(0%)
Intermittent clamp/release regimen	24	(2%)
Intermittent catheterization	11	(1%)
Pharmacotherapies		
Distigmine	151	(16%)
Carbachol/bethanecol/urecholine	55	(6%)
Tranquillizers (benzodiazepines/alcohol)	22	(2%)
Prostaglandins	12	(1%)
Alpha-adrenoreceptor blockers	6	(1%)
Phenazopyridine	3	(0%)
Mist. Pot. Cit.	2	(0%)
Anti-spasmodics (flavoxate/emepronium/imipramine)	26	(3%)
Diuretics	2	(0%)
Mazindol	1	(0%)
Physical treatments		
Urethral dilatation	65	(7%)
Urethrotomy	2	(0%)
Bladder training	8	(1%)
Pelvic floor exercises	5	(0%)
Faradism	2	(0%)
Cystometry	10	(1%)
Discharge with catheter *in situ*	29	(3%)

has also been shown to speed the return of spontaneous voiding following incontinence surgery, presumably by allowing the more rapid resolution of peri-urethral oedema (Andersen *et al.* 1982).

The selection of urethral catheter types was perhaps the most consistent area of practice examined. The infrequent use of female length catheters by a group of clinicians dealing almost exclusively with female patients is somewhat surprising. The use of the standard male length catheter in a female patient makes the device more difficult to disguise under clothing, and easier to pull accidentally: the additional length also gives increased potential for kinking and blockage (Norton 1986).

In the prophylaxis against catheter-associated infection, there were no differences in practice between those with a major urological interest and their 'generalist' colleagues; there were, however, consistent age-related trends in the use of antibiotics and bladder irrigation. Whether this trend reflects a tendency to increasing use of these methods with increasing experience in the speciality, or to their less frequent use by those more recently trained, cannot be answered from these data; the latter, however, would seem the more likely. The literature regarding the place of prophylactic chemotherapeutic agents in catheterized patients remains contradictory. In the case of long-term catheterization there is general agreement that prophylaxis is inadvisable because of the risks of persistent colonization and emergence of resistant organisms (Kunin 1979; Turck & Stamm 1981). The same also applies to the therapeutic use of antimicrobials in patients documented to have bacteriuria, but who remain asymptomatic. In the case of short-term catheterization, particularly in the postoperative situation, prophylactic antibiotics may be used for two distinct purposes: to prevent postoperative bacteriuria in patients with sterile urine before operation and to prevent septicaemia in those who come to operation with infected urine. In the first situation there have been several studies which have documented a reduction in postoperative bacteriuria with several antimicrobial agents, e.g. nitrofurantoin, amoxycillin, co-trimoxazole, and cephalosporins. These have been reviewed recently by Slade & Gillespie (1985) who concluded that since postoperative bacteriuria is easy to treat, and rarely causes serious harm, the routine use of antibiotics is likely to do more harm than good. Where patients come to surgery with already infected urine, the literature is again inconsistent, and in a recent review Hirschman & Inui (1980) had to conclude that the value of administering antibiotics to patients in this situation remains uncertain. It would seem therefore that antibiotic therapy should perhaps be reserved for those patients who develop signs and symptoms referrable to urinary tract infection, or whose catheter is likely to be removed within a few days of the recognition of bacteriuria.

Kass & Schneiderman (1957) showed that bacteria may gain entry to the urinary tract via the peri-catheter route, and despite many changes in catheter management, this remains an important source of infection (Schaeffer & Chmial 1983; Daifuku & Stamm 1984). Studies on the efficacy of meatal toilet regimes using povidone-iodine, non-antiseptic soap (Burke *et al.* 1981) and polyantibiotic ointment (Burke *et al.* 1983) have shown little or no benefit; indeed it has been suggested that such methods may actually be hazardous and encourage infection in high-risk groups. The use of regular bladder irrigations or washouts using neomycin, chlorhexidene, and noxythiolin have similarly been advocated as a means of reducing clinical infection rates. Brocklehurst & Brocklehurst (1978) found them to have little influence, however, and antibacterial solutions at least may run the risk of inducing resistance (Warren *et al.* 1978). Although uncontrolled retrospective studies have claimed clinically significant beneficial effects from the instillation of antiseptic agents into catheter drainage bags (Southampton Infection Control Team 1982; Desautels & Lang 1982), controlled studies suggest that whilst the bag itself can be effectively sterilized by instillation of hydrogen peroxide (Thompson *et al.* 1984) or chlorhexidine (Gillespie *et al.* 1983), the frequency of urinary infection is unchanged.

Whilst these measures remain, in some cases, quite widely practised by gynaecologists in the British Isles, their value in the prevention of catheter-associated infection is clearly unproven.

Bypassing, or leakage of urine around a urethral catheter, is a very common problem in long-term catheterized patients (Brocklehurst & Brocklehurst 1978; Ferrie *et al.* 1979), and it may occur in the short term also. Practices described in this study show wide variation, and some consideration of the underlying mechanism of the problem is perhaps justified. The simplest explanation is kinking or twisting of the catheter or drainage tubing, and this should be investigated immediately. If no site of occlusion is evident externally, the catheter should be gently flushed to relieve possible blockage by clot or mucosal slough. If this does not ease the problem the catheter should be removed and examined for signs of encrustation; if present, then in the long-term catheterized patient at least, it may be worth changing to an all-silicone catheter. In the absence of encrustation the most likely cause of bypassing is the occurrence of uninhibited detrusor contractions; if the catheter cannot be removed a course of anti-cholinergic drugs, e.g. probanthine or oxybutinin, is often beneficial. The problem is certainly exacerbated by large balloon catheters, and the temptation to increase the volume in the balloon in an effort to occlude the bladder neck must be avoided (Norton 1986). Similarly, the habit of using catheters of progressively increasing calibre is to be eschewed, since this will make the urethra more and more patulous, and increase the risk of urethritis and paraurethral abscess formation (Blandy 1981).

The practice of intermittent clamping and releasing of the urethral catheter in an effort to 'regain bladder tone' remains widespread, being employed by 55% of gynaecologists surveyed (although it is less commonly used by those with a major interest in urology). Although there have been reports that the technique may encourage spontaneous voiding following bladder neck surgery (Segal & Corlett 1979), it is somewhat illogical, and there are potential dangers. First, it does not achieve its expressed aim, and will actually reduce bladder tone. Second, on the busy ward, 4 h so easily becomes 6 or 8 h, with consequent danger of overdistension. This will not only encourage urinary stagnation and infection, but may lead to long-term impairment of bladder contractility (Wein & Levin 1982).

The technique of clean intermittent self-catheterization was introduced by Lapides *et al.* (1972) in patients with outflow obstruction; whilst its major role is undoubtedly in the management of adults and children with neuropathic bladders, it has also a definite place in women with chronic voiding dysfunction (Murray *et al.* 1984). Whilst the general gynaecologist would not be expected to have much use for the technique, and indeed it is perhaps best managed by the specialist gynaecological urologist, with specialist nursing back-up, it is somewhat surprising that only one in three of those with a major specialist interest in urology sees any place within their practice for this form of self-catheterization.

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I should like to thank the many members and fellows of the RCOG who took the time and trouble to complete and return questionnaires; in particular I would thank and apologise to those in Scotland and Ireland who went to this effort despite my unforgivable omission of their health regions from the document circulated. My thanks are due to Dr Adrian Grant of the National Perinatal Epidemiology Unit in Oxford, and Mr Stuart Stanton of St George's Hospital Medical School, London, for their helpful comments on the analysis and presentation of the data. The financial assistance of Becton-Dickinson UK Ltd in the circulation of questionnaires is gratefully acknowledged.

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The distribution of CA 125 in the reproductive tract of pregnant and non-pregnant women

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Summary. Investigation of serum and tissue homogenates obtained from first, second and third trimester pregnancies, and from non-pregnant women, has provided further insight into the possible origin of the CA 125 antigen. Serum CA 125 levels were higher in the first trimester (median 53·6 U/ml, range 15·6–268·3 U/ml) than in non-pregnant women (median 19·3 U/ml, range 7·2–27·0 U/ml) and later in pregnancy (second trimester: median 18·5 U/ml, range 12·0–25·1 U/ml, third trimester: median 19·2 U/ml, range 16·8–43·8 U/ml) ($P<0{\cdot}05$) but were two orders of magnitude less than in second trimester amniotic fluid (median 4825 U/ml, range 3200–9300 U/ml). Fetal serum CA 125 activity was consistently <20 U/ml. The highest tissue levels of CA 125 were detected in first trimester decidual homogenate (median 4547 U/100 mg, range 340·4–20 851 U/100 mg) and were greater than in non-pregnant endometrium (median 388 U/100 mg, range 100·9–3341 U/100 mg) ($P<0{\cdot}01$) and term decidua (median 116 U/100 mg, range 32·7–449·9 U/100 mg) ($P<0{\cdot}01$). These observations suggest that CA 125 is synthesized by normal endometrium and decidua and that increased CA 125 activity during pregnancy is of decidual origin.

CA 125 is a tumour-associated antigen recognized by a monoclonal antibody, OC125, which was produced following immunization of mice with an ovarian cancer cell line OVCA 433 (Bast *et al.* 1981). Using a radioimmunoassay to detect CA 125 in serum, Bast *et al.* (1983) reported that 82% of patients with ovarian carcinoma had levels > 35 U/ml compared with 1% of apparently healthy individuals. Subsequent work has confirmed the value of serum CA 125 measurement in monitoring the treatment of ovarian cancer (Canney *et al.* 1984; Dodd *et al.* 1985; Brioschi *et al.* 1987; Cruickshank *et al.* 1987) and its role as a screening test for early stage ovarian cancer is currently under investigation (Jacobs *et al.* 1988).

Recent studies have demonstrated that elevation of serum CA 125 activity is not specific to ovarian cancer. In addition to several other malignant conditions, serum CA 125 levels may be elevated in endometriosis (Barbieri *et al.* 1986; Pittaway & Fayes 1986), pelvic inflammatory disease (Halila *et al.* 1986) and pregnancy (Niloff *et al.* 1984; Halila *et al.* 1986; Seki *et al.* 1986). High levels of CA 125 activity have also been reported in amniotic fluid (Niloff *et al.* 1984; O'Brien *et al.* 1986), cervical and endometrial fluid (de Bruijn *et al.* 1986), and decidua and chorion at term (O'Brien *et al.* 1986).

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The origin of the CA 125 antigen in ovarian cancer is likely to be the tumour itself, but the primary site of synthesis in the genital tract and in pregnancy is unclear. This study was undertaken as part of a systematic analysis of the distribution of CA 125 activity in serum and tissues of the female reproductive tract in the pregnant and non-pregnant state.

Patients and methods

Patients and samples

Non-pregnant. Proliferative (n=7) and secretory (n=5) endometrium was obtained from premenopausal women undergoing dilatation and curettage or hysterectomy for benign conditions. Venous blood was obtained from the same women immediately before operation. Myometrium was obtained from the four patients undergoing a hysterectomy.

First trimester pregnancy. Matched samples of decidua (n=10), trophoblast (n = 9), fetal membranes (n=9) and maternal venous blood were obtained from 11 women undergoing elective termination of pregnancy at 8 to 13 weeks gestation (according to menstrual data).

Second trimester pregnancy. Maternal and fetal blood samples and amniotic fluid were obtained at the time of diagnostic fetoscopy (Stabile *et al.* 1988) from seven retrospectively defined normal pregnancies (18–21 weeks gestation).

Third trimester pregnancy. Matched samples of decidua (n=8), trophoblast (n=9), fetal membranes (n=8), amniotic fluid (n=8), cord blood (n=8) and maternal venous blood (n=8) were obtained at term from 10 women who underwent a normal vaginal delivery or an elective caesarean section at term after an uneventful pregnancy.

Amniotic fluid and venous blood samples were centrifuged at 3000 r/min for 10 min after clotting at room temperature and the serum separated and aliquoted. Tissue samples were obtained by naked eye dissection as described in detail elsewhere (Rutanen *et al.* 1986*a*, *b*) and a representative sample of the tissue obtained was sent for histological examination to confirm the tissue type Samples were washed in 0·5 M saline and snap frozen in liquid nitrogen in the operating theatre, before storage at −20°C. Stored tissue samples were homogenized in phosphate-buffered saline, pH 7·4, containing 0·05 M azide, triton X-100 1% and 175 μg/ml phenyl-methyl-sulphonyl fluoride (Sigma) (50 mg tissue/ml), centrifuged at 10 000 *g* for 15 min at 4°C and the supernatant separated and aliquoted. All samples were stored at −20°C until assayed for CA 125 activity.

Radioimmunoassay

CA 125 activity in serum, tissue and amniotic fluid samples was measured by radioimmunoassay (Abbott Laboratories, Chicago, USA). The detection limit of the assay was 7 U/ml. The intra-assay coefficients of variation at 30 U/ml and 200 U/ml were 7·6% and 8·2% respectively and the inter-assay coefficients of variation at the same levels were 8·4% and 9·1% respectively. The tissue content of CA 125 activity was expressed as the amount per 100 mg wet weight.

Gel filtration

Size chromatography was performed using an ACA-34 gel filtration column (104 cm length and 1 cm diameter equilibrated with 10 mM Tris-HCl, pH 7·4, 5 mM azide). One-millilitre samples were applied and the column run at 8 ml/h for the collection of 1-ml fractions. The void volume as determined by the elution of blue dextran 2000 was 36 ml.

Statistical methods

Statistical analysis was performed using Wilcoxon's sum of ranks test. The upper limit of normal for serum CA 125 levels adopted in this study was 35 U/ml (Bast *et al.* 1983).

Results

CA 125 activity in first trimester decidual homogenate, endometrial homogenate, amniotic fluid and ovarian cancer diluted in parallel with assay standards. CA 125 activity in the same samples eluted with blue dextran in the void volume of an ACA 34 gel filtration column (exclusion limit 750 kD).

Non-pregnant specimens (Fig. 1). Serum CA 125 activity in all samples from non-pregnant women was <35 U/ml (median 19·3 U/ml, range 7·2–27·0 U/ml). CA 125 levels in homogenates of non-pregnant endometrium ranged from

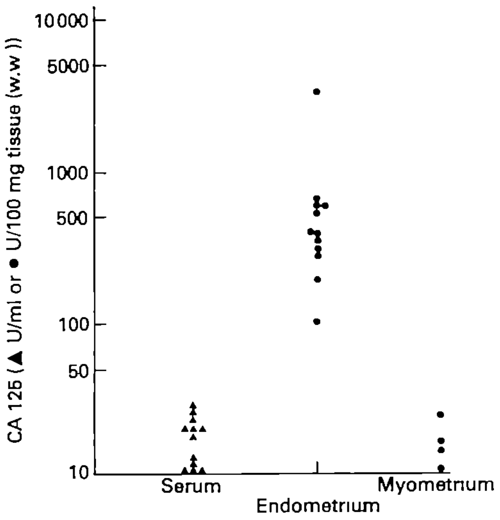

Fig. 1. CA 125 activity in matched serum (n=12), endometrium (n=12) and myometrium (n=4) from 12 women.

100·9 U/100 mg to 3341 U/100 mg (median 388·0 U/100 mg) and were higher than in myometrium (median 32·1 U/100 mg, range 14·2–49·9 U/100 mg) (P<0·01). There was no significant difference apparent between CA 125 activity in homogenates of proliferative and secretory endometrium.

First trimester pregnancy (Fig. 2). Serum CA 125 levels exceeded 35 U/ml in 8 of 11 women (median 53·6 U/ml, range 15·6–268·3 U/ml). Tissue levels of CA 125 in first trimester decidua (median 4547 U/100 mg, range 340·4–20 851 U/100 mg) were greater than in fetal membranes (median 122·6 U/100 mg, range 15·0–611·5 U/100 mg) (P<0·01) and trophoblast (median 60·5 U/100 mg, range 14·6–720 U/100 mg) (P<0·01).

Second trimester pregnancy (Fig. 3). Maternal serum levels in the second trimester pregnancies did not exceed 35 U/ml (median 18·5 U/ml, range 12·0–25·1 U/ml). Fetal serum CA 125 levels were consistently <10 U/ml. By contrast, CA 125 levels in second trimester amniotic fluid samples were two orders of magnitude higher (median 4825 U/ml, range 3200–9300 U/ml).

Third trimester pregnancy (Fig. 4). Maternal serum CA 125 levels were <35 U/ml in 7 of 8 samples (median 19·2 U/ml, range 16·8–43·8 U/ml). Fetal serum levels were all <20 U/ml and less than maternal serum levels (P<0·01). CA 125 activity in decidua (median 116·5 U/100 mg, range 32·7–449·9 U/100 mg) was less than in fetal membranes (median 757·4 U/100 mg, range 399–1452 U/100 mg) (P<0·01), whilst all trophoblastic specimens obtained at term contained <10 U/100 mg CA 125 activity. CA 125 activity in amniotic fluid was 10-fold greater than in maternal serum (median 315·5 U/ml, range 313–463 U/ml).

The highest tissue levels of CA 125 activity were detected in first trimester decidual homogenate and were greater than in non-pregnant endometrium (P<0·01) and term decidua (P<0·01). Serum CA 125 levels were higher in specimens obtained during the first trimester of pregnancy than in the second trimester (P<0·01), third trimester (P<0·05) or from non-pregnant women (P<0·05). CA 125 activity in amniotic fluid was higher in the second trimester than at term (P<0·002).

Discussion

The elution profile of CA 125 from the tissues and fluids examined suggests that the CA 125 determinant is expressed in molecules with a molecular weight >750 kD. This is consistent with previous reports (Davis *et al.* 1986; O'Brien *et al.* 1986). CA 125 activity in the different compartments demonstrated parallelism on dilution providing further evidence of immunochemical identity.

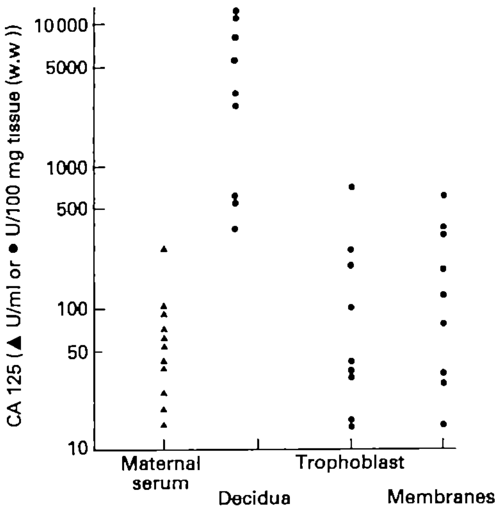

Fig. 2. CA 125 activity in matched maternal serum and fetal and maternal tissue extracts from 11 first trimester pregnancies.

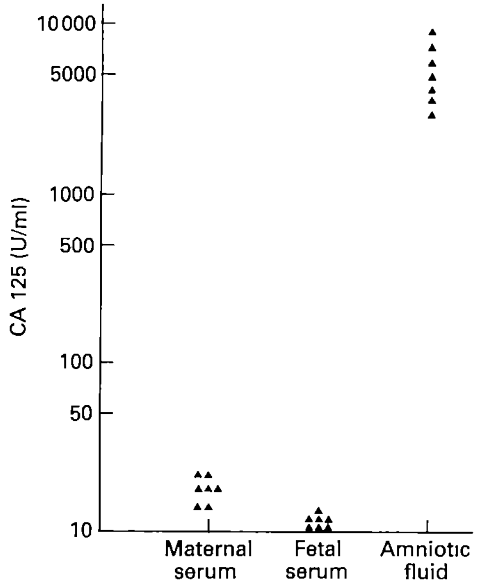

Fig. 3. CA 125 activity in matched maternal and fetal serum and amniotic fluid from seven second trimester pregnancies.

Previous studies have described a significant increase in maternal serum CA 125 levels during the first trimester of pregnancy compared with the non-pregnant state or later in pregnancy and high levels of CA 125 in amniotic fluid (Niloff *et al.* 1984; Halila *et al.* 1986; Seki *et al.* 1986; O'Brien *et al.* 1986). Our results are consistent with these previous observations, but in addition we found high levels of CA 125 activity in normal endometrium and in first trimester decidua.

The origin of CA 125 activity in pregnancy has not yet been established. Although a fetal origin has been suggested (Halila *et al.* 1986; Seki *et al.* 1986), O'Brien *et al.* (1986) detected only low levels of the antigen in cord blood and neonatal urine specimens. Our findings do not support a fetal origin for CA 125 (Figs. 3 & 4). The high levels of CA 125 activity seen in first trimester decidua in association with increased maternal serum levels raise the possibility that the source of CA 125 activity in pregnancy is the decidualized endometrium. This suggestion is supported by Bischof *et al.* (1986) who have reported a reduction in CA 125 content of tissue and media in cycloheximide-treated in-vitro cultures of first trimester decidua compared to controls. Further circumstantial evidence of a decidual origin for CA 125 is the striking similarity between the compartmental distribution of CA 125 and that of placental protein 14 (synonyms: progestagen-dependent endometrial protein, alpha uterine protein, pregnancy-associated endometrial alpha 2 globulin), a protein of endometrial/decidual origin (Bell 1986). The highest serum and decidual tissue levels of both CA 125 and PP14 have been observed in the first trimester whilst the highest amniotic fluid levels were seen in the second trimester (T. Fay, personal communication).

The high levels of CA 125 activity detected in endometrium here are also consistent with the hypothesis that CA 125 is a product of the normal endometrium/decidua. Further evidence for the endometrial origin of CA 125 is the observation of high CA 125 activity in endometrial and cervical mucus (de Bruijn *et al.* 1986). The high endometrial levels of CA 125 in women with low serum CA 125 activity suggests that CA 125 is not normally secreted into the peripheral circulation and that there may be a barrier to CA 125 between the normal endometrium and the circulation. The reason for elevation of serum CA 125 levels at the time of menstruation in a small group of women is unclear, although tubal reflux with absorption of CA 125 via the peritoneum has been suggested (Mastropaolo *et al.* 1986).

Our preliminary results suggest that CA 125 is a product of normal endometrium and decidua, and in-vitro studies are currently underway to investigate *de novo* synthesis of CA 125 in these tissues. Further clinical studies are in progress to

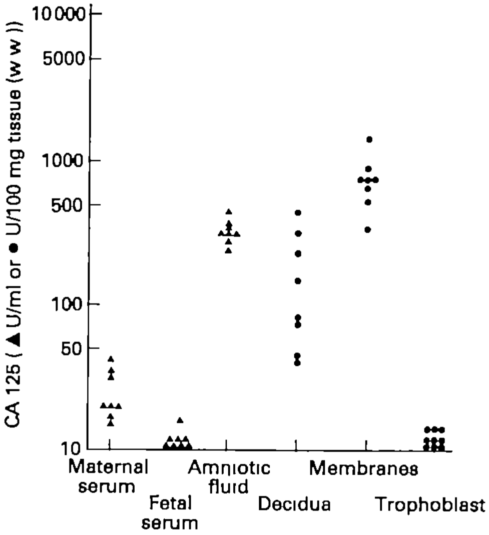

Fig. 4. CA 125 activity in matched maternal and fetal serum and tissue extracts from 10 full-term pregnancies.

observe the variation of serum CA 125 during early pregnancy and its complications.

Acknowledgments

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Malignant lymphoma presenting as initial symptom in the uterus. Case report

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Case report

The patient was a 46-year-old married woman (gravida 8, para 6) with a regular menstrual cycle. In May 1986, she began to have a brownish discharge subsequent to the insertion of an intrauterine contraceptive device. On 12 June she was seen at another hospital as she had had persistent and increasing vaginal blood loss for a month. Laboratory findings were negative. The uterus was the size of a goose egg, and tissue obtained by curettage consisted of malignant lymphoma.

On 11 August the patient was referred to the Osaka Medical College Hospital. A scintigram using ^{67}Ga showed a hot nodule at the bottom of the pelvic cavity, and several in other parts of the body (Fig. 1). No abnormal lymphoid cells were found in the peripheral blood. Laboratory findings before the surgery reported the following: red blood count $3{\cdot}64\times10^6$/ml; white blood count 3770/ml with 53·1% neutrophils, 7·0% monocytes, 1·7% eosinophils, 0·4% basophils, 36·5% lymphocytes; lactic dehydrogenase (LDH) 1622 units/l (1:9%, 2:37%, 3:40%, 4:12%, 5:2%); glutamate-oxaloacetate transaminase (GOT) 10 units/l; glutamate pyruvate transaminase (GPT) 6 units/l; blood urea nitrogen (BUN) 11 mg/dl; IgG 896 mg/dl; IgA 160 mg/dl; IgM 61 mg/dl; Cu 139 µg/dl. The serum levels of tumour markers (CA125, CA19-9, CEA and AFP) were not elevated.

A total hysterectomy and right salpingo-oophorectomy were performed. There was bilateral enlargement of the internal iliac nodes and obturator nodes However, careful microscopic examination failed to demonstrate abnormal lymphoid cells in the uterus, the right ovary or the lymph nodes. After surgery the red blood count was $3{\cdot}49\times10^6$/ml; white blood count 4740/ml with 67·4% neutrophils, 7·2% monocytes, 3·1% eosinophils, 0·6% basophils, 20·3% lymphocytes; LDH 880 units/l. Chemotherapy was not considered necessary and the patient returned home on 14 October.

On 4 November the patient noticed a swelling in the vagina and consulted our hospital again. The tumour was 8×8×2 cm, and a biopsy was shown to consist entirely of lymphoma cells. Chemotherapy (vincristine, endoxan, adriacin and predonin) was started on 13 November. After one course of chemotherapy lymphoma cells showed some degeneration but were still present. The vaginal tumour was irradiated with a radium needle to a total of 2000 rad, and chemotherapy was continued.

In January 1987, the left subclavian and other lymph nodes were enlarged and tumour cells had replaced 90% of the cells in the bone marrow and 50% of those in the peripheral blood. Chemotherapy was started again, but bleeding increased. The patient died on 20 January. Laboratory findings before death were as follows: red blood count $2{\cdot}70\times10^6$/ml; white blood count 18 290/ml with 32·0% neutrophils, 5·0% monocytes, 3·5% eosinophils, 0·5% basophils, 7·0% lymphocytes, 44·5% others; LDH 32 940 units/l.

Pathology report

Biopsy findings

The endometrial stroma showed diffuse infiltration with atypical cells (Fig. 2). These had a

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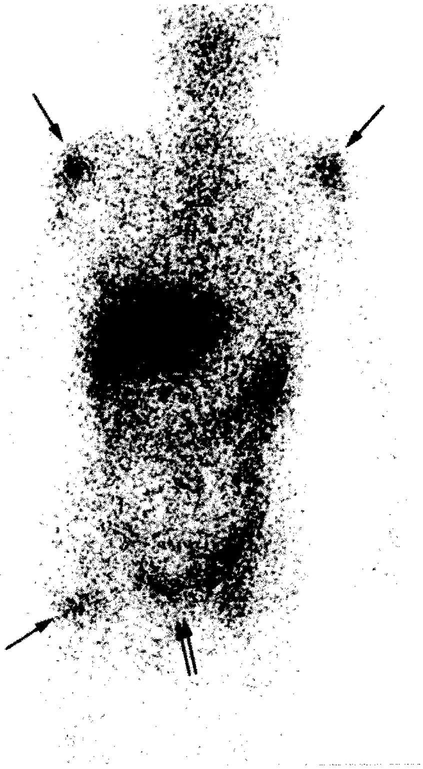

Fig. 1. A hot nodule in the pelvic cavity (double arrow) and several in other parts (single arrows) were shown by ^{67}Ga-scintigram.

round to oval, large nucleus and scanty cytoplasm, and showed many mitotic figures. Small but distinctive nucleoli were situated at the nuclear membrane. The histological diagnosis was non-Hodgkin's lymphoma, diffuse, large-cell type consisting mostly of non-cleaved cells as described by The Non-Hodgkin's Lymphoma Pathologic Classification Project (1982). Immunoperoxidase reactions with antibodies to IgA, IgG, IgM kappa chain and lambda chain were all negative in the lymphoma cells.

Autopsy findings

The pelvic cavity was occupied by a tumour mass in which the left ovary, intestines and the mesentery were involved. The tumor mass involved the vagina and the bladder. The wall of the vagina was firm and pale. Numerous tumour nodules were found all over the body. Swelling of the lymph nodes was not prominent.

Microscopically the tumours consisted of diffuse proliferation of lymphoma cells. They were similar to the cells seen in the biopsy, but were more atypical and unequal in size. Mitotic figures were frequent. The lymph nodes had lost their structure because of the proliferation of lymphoma cells.

Discussion

Freeman *et al.* (1972) reported that primary extranodal malignant lymphoma accounted for about 25% of malignant lymphoma, excluding Hodgkin's disease. They reported eight tumours which arose in the uterus. Fox & More (1965) and Wright (1973) reported their own cases, and defined criteria for the diagnosis of primary extranodal lymphoma. They accepted as primary uterine lymphoma only those tumours fulfilling the following criteria: (1) at the time of initial diagnosis, the disease process was clinically confined to the uterus, (2) full investigation failed to reveal any evidence of disease elsewhere in the body, (3) the peripheral blood cells showed no evidence of leukaemia, and (4) if further lymphomatous deposits occurred at sites removed from the genital tract, then a time interval of several months should have elapsed between the appearance of primary and secondary tumours. In our patient, scintigraphy after the initial biopsy and before the hysterectomy showed several hot nodules in the pelvic cavity and at other sites. The disease recurred 5 months after the initial diagnosis. For these reasons, we consider that our patient had a malignant lymphoma presenting with uterine bleeding and not a primary uterine lymphoma. The malignant lymphoma of diffuse, large-cell type as seen in our patient is classified as intermediate grade malignancy by The Non-Hodgkin's Lymphoma Pathologic Classification Project (1982). However, its median survival time is 1·5 years, and almost equivalent to that of immunoblastic type or lymphoblastic type in high-grade malignancy.

It is of interest that the endometrial lesion was completely removed by the curettage because the uterus has been reported to be heavily infiltrated with lymphoma cells, regardless of whether the lesion was primary or secondary (Harris & Scully 1984). Complete disappearance of lymphoma cells in our patient was confirmed by a careful microscopic examination of the surgical specimens. It was not until the terminal stage of the disease when it became leukaemic

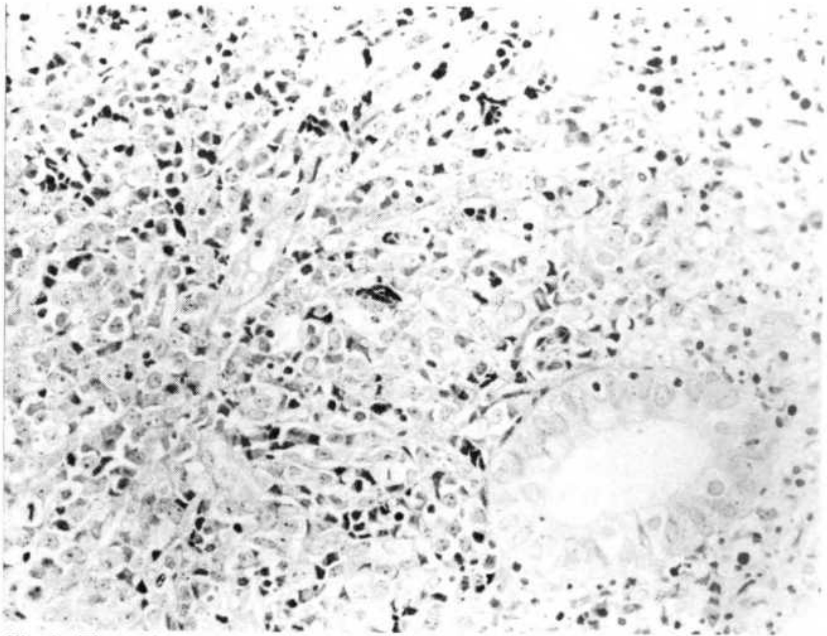

Fig. 2. The endometrium was heavily invaded by lymphoma cells (haematoxylin and eosin ×300).

that the involvement of lymph nodes was clinically obvious.

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Pneumococcal neonatal colonization and sepsis in association with maternal genital pneumococcal colonization. Case reports

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Case reports

Patient 1

A 22-year-old woman, in her second pregnancy, was delivered on 10 May 1984 at 38 weeks gestation of a live female infant weighing 2·55 kg. The baby was in good condition at birth and the membranes had been ruptured for only 27 min before delivery. On the second day of life the baby developed signs of meningitis and was given intravenous ceftazidime and penicillin after bacterial cultures had been taken, but she deteriorated rapidly and died at 72 h of age. *Streptococcus pneumoniae,* sensitive to penicillin, tetracycline, erythromycin and ceftazidime, was isolated from blood and CSF cultures and from eye and nasal swabs. At post-mortem the infant was found to have a purulent meningitis.

The mother of the baby had no problems during pregnancy and was well and apyrexial throughout labour. Genital swabs taken 3 days after delivery revealed genital colonization with *S. pneumoniae.*

Patient 2

On 8 January 1985 a 20-year-old woman in her first pregnancy was delivered at 39 weeks gestation of a male infant weighing 3·035 kg. This was a breech delivery, and there was evidence of perinatal asphyxia with meconium-stained amniotic fluid. The baby had an Apgar score of 5 at 1 min and spontaneous respirations were established at 2 min. Because the baby was slightly cyanosed at 5 min he was admitted to the special care baby unit for observation and given oxygen by headbox for about 2 h. The baby developed two episodes of asymptomatic hypoglycaemia in the first 7 h of life which responded to intravenous dextrose, and 6 h later he was tolerating oral feeds well and his blood sugar was normal. On the second day of life the baby developed signs of septicaemia and meningitis and had bilateral pneumonitis on the chest radiograph. After bacterial cultures were taken the baby was given intravenous penicillin and tobramycin, and later chloramphenicol, but despite ventilatory support the baby deteriorated and died at 72 h. *Streptococcus pneumoniae* was isolated from blood and CSF cultures. Post-mortem confirmed the clinical findings of pneumonia and a purulent meningitis.

S. pneumoniae was subsequently isolated from genital swabs taken from the mother 3 days after delivery. Both isolates from the mother and baby were sensitive to penicillin, erythromycin and chloramphenicol. The mother was well and apyrexial throughout.

Patient 3

This male baby was born on 9 August 1985 after a normal vaginal delivery at 39 weeks gestation. He was clinically unwell in the first few hours of life and was given intravenous penicillin and tobramycin after blood cultures and surface swabs were obtained. The baby responded rapidly to antibiotics and is well. *S. pneumoniae,* sensitive to penicillin, erythromycin and chloramphenicol, was isolated from blood culture

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and nose, throat and eye swabs. This infant's mother was pyrexial during labour and was subsequently found to have genital colonization with *S. pneumoniae*. She was given penicillin and her fever settled.

Patient 4

This patient had prolonged rupture of membranes and a high vaginal swab showed colonization with *S. pneumoniae*. She was treated with penicillin before delivery. Her infant was born vaginally on 4 February 1986 at 39 weeks gestation and although asymptomatic at birth the baby was given penicillin after blood culture and surface swabs had been taken. *S. pneumoniae* was isolated from nose, eye and throat swabs only. The baby remained well and asymptomatic.

Discussion

Endometritis and neonatal sepsis due to *Streptococcus pneumoniae* has been described by McCarthy & Cho (1979) and Hutchinson *et al.* (1984), but it is not considered a common cause of infection, either in the newborn infant or in the puerperal woman. The early onset of the neonatal infection in our patients with no evidence of preceding infection in the mothers on admission suggests an ascending rather than transplacental infection.

The genital tract is not recognized as a carriage site for pneumococci, although pneumococcal infection of the genital tract especially during pregnancy and in the absence of any other site has been reported by Nuckols & Hertig (1938) and Duff & Gibb (1983). Recent changes in practising sexual activities (oro-genital) may alter the ecology of genital tract microbial flora and may result in colonization of the genital tract with upper respiratory tract bacteria such as *S. pneumoniae* and *Haemophilus* species. This may lead to a new spectrum of neonatal or puerperal infection.

Clinicians and microbiologists should be aware of this association so that if a newborn infant develops signs of infection, *Streptococcus pneumoniae* should be considered as a possible causal organism derived from the mother's vaginal tract.

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Fetal high output cardiac failure and acute hydramnios caused by large placental chorioangioma. Case report

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Case report

A 31-year-old woman was admitted to the high-risk obstetric unit in the 25th week of her third pregnancy, because of rapidly developing hydramnios. Her two previous pregnancies had resulted in normal deliveries. The present pregnancy was uneventful up till 22 weeks gestation. Ultrasound studies at that time revealed a single normal fetus, with an excessive amount of amniotic fluid. The placenta was located posteriorly, and contained an 8×8×9 cm solid and partially cystic mass (Fig. 1), compatible with a placental chorioangioma.

During the next 2 weeks, the patient complained of rapid increase in the size of her abdomen, severe dyspnoea, backache and uterine contractions. Upon examination, the uterine size corresponded to 40 weeks gestation. Ultrasonography disclosed severe hydramnios and a normal fetus, whose size was appropriate for gestational age. The placental mass was unchanged.

Two-dimensional direct M-mode echocardiography with Doppler analysis revealed normal fetal cardiac anatomy, persistent sinus tachycardia (170–180 beats/min) and a moderate amount of pericardial effusion (Fig. 2). Pulsed Doppler echocardiography (PDE) was performed, the fetal cardiac output, calculated as described by Wladimiroff *et al.* (1984) and Maulik *et al.* (1985), was 660 ml/min in the pulmonary root, and 440 ml/min in the ascending aorta. Both values are much higher than the mean values at 25 weeks gestation (Wladimiroff *et al.* 1984; Maulik *et al.* 1985). Umbilical blood flow was normal and the systolic to diastolic ratio was <2. No signs of fetal hydrops were observed, therefore no treatment was initiated. Fetal cardiac functions were assessed twice a week and no deterioration was observed.

Three weeks later, the hydramnios decreased gradually, the placental mass size reduced to 6×6×7 cm, fetal growth was normal, the pericardial effusion disappeared and the cardiac output was 220 ml/min in the pulmonary root, and 150 ml/min in the ascending aorta. Both values were appropriate for the gestational age.

At 36 weeks gestation, a normal female infant was born with a birthweight of 2550 g and 1- and 5-min Apgar scores of 9.

The placenta weighed 600 g with a centrally located umbilical cord and yellowish fetal membranes. A yellowish-pink well-demarcated soft mass, 5·5 cm in diameter, was found eccentrically placed on the maternal surface. It was easily separated from the adjacent placenta and membranes, disclosing a vascular pedicle (Fig. 3). Microscopic examination revealed a necrotic calcified chorioangioma in an otherwise normal placenta of the third trimester (Fig. 4).

Discussion

Chorioangioma constitutes the most common neoplasm of the placenta and its frequency varies from 0·01% (Marchetti 1939) to 1% (Wentworth 1965). The tumour is most probably a hamartoma of the primitive chorionic mesenchyme (Pritchard *et al.* 1985).

The clinical implications of such tumours, especially of large size, include: acute hydramnios (McInroy & Kelsey 1954), micro-

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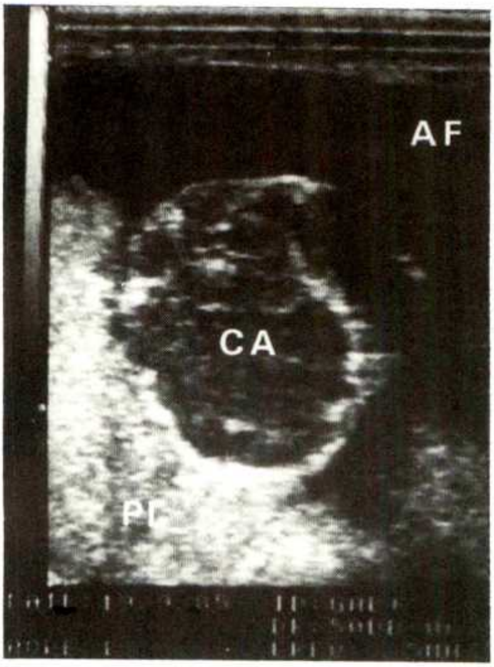

Fig. 1. Large solid and partially cystic placental chorioangioma. AF, Amniotic fluid; CA, chorioangioma; PL, placenta.

angiopathic haemolytic anaemia with fetal hydrops (Jones *et al.* 1972), intrauterine growth retardation (Hurwitz *et al.* 1983; Wallenburg 1971), fetal iron deficiency anaemia (Hurwitz *et al.* 1983), fetal high output cardiac failure (Benson & Joseph 1961; Wallenburg 1971), severe fetal distress (Hurwitz *et al.* 1983) and intrauterine fetal death (Knoth *et al.* 1976).

Hydramnios constitutes the most frequently reported clinical complication associated with chorioangioma, and is reported to occur in 18–35% of all cases (Asadourian & Taylor 1968; Kuhnel 1933; Siddall 1924). The reason for the development of hydramnios is still a matter of controversy. Several hypotheses have been suggested: (i) mechanical obstruction to blood flow by a tumour localized near the cord insertion, causing greater transudation of fluid (Wallenburg 1971), (ii) increased transudation of fluid through the large vascular surface of the tumour which bulges towards the fetal surface (Kotz & Kaufman 1939), and (iii) functional insufficiency of the placenta caused by by-passing of the fetal blood into the vascular tumour which stimulates an increased excretion of waste metabolites by the fetal kidneys (McInroy & Kelsey 1954).

Benson & Joseph (1961) suggested that chorioangioma can be considered as an arteriovenous shunt in the systemic fetal circulation. Their suggestion was strengthened by Reiner & Fries (1965) who demonstrated the presence of such a shunt in chorioangioma by means of postpartum injection of the placenta with a radio-opaque substance. This hypothesis offers a possible explanation, not only for the hydramnios, but also for the other clinical com-

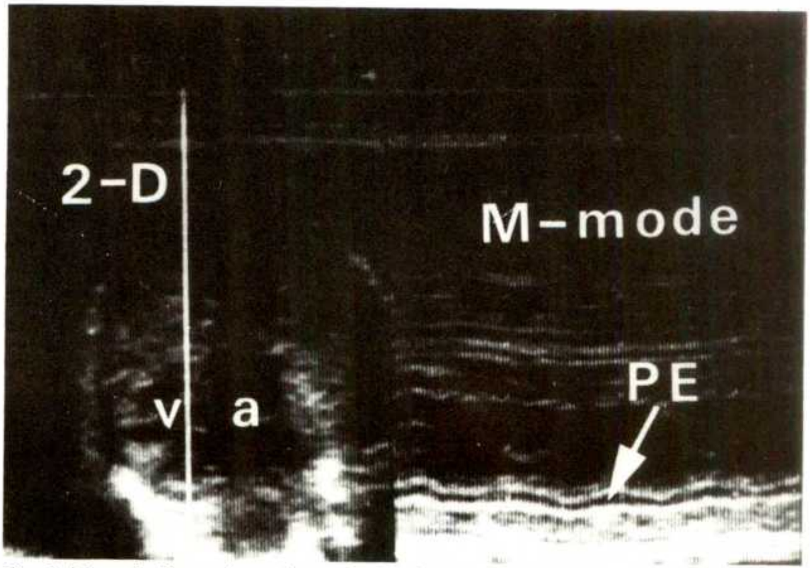

Fig. 2. M-mode directed two-dimensional echocardiography of the fetal heart demonstrating pericardial effusion. v, Ventricles; a, atria; PE, pericardial effusion.

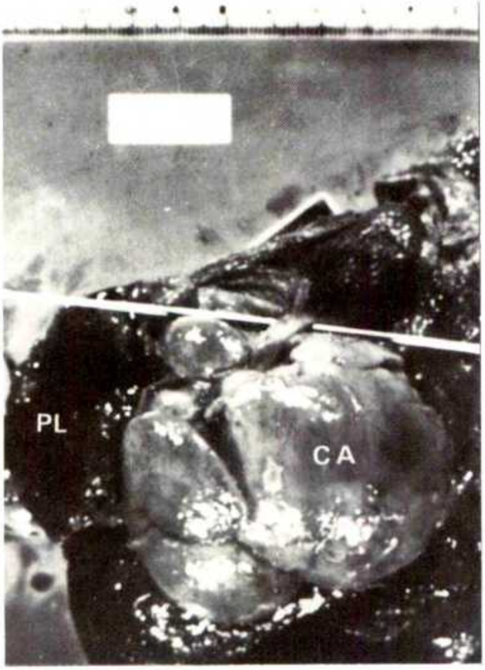

Fig. 3. A well-demarcated necrotic chorioangioma, 5·5 cm in diameter, located on the maternal surface of the placenta. The probe passes under the supplying artery. PL, Placenta; CA, chorioangioma.

plications often associated with placental chorioangioma.

Based on our understanding of the physiological effects of a systemic arteriovenous shunt in postnatal life, the presence of such a shunt in fetal life will result in an increase in cardiac output and venous return. In the presence of a sufficiently large shunt volume, cardiomegaly and cardiac failure may develop. Such shunt volume depends on the size and number of vascular communications in the tumour. The fetus and the newborn are especially prone to cardiac failure, since even a moderate absolute shunt volume causes a high ratio of shunt blood to total blood volume. Cardiac hypertrophy and failure have been reported in cases of cerebral, hepatic, cutaneous and multiple haemangiomas in the neonatal period (Wallenburg 1971).

Benson & Joseph (1961) reported a neonatal death due to congestive heart failure without any obvious cause, other than the presence of a large chorioangioma. Wallenburg (1971) reported a newborn with cardiomegaly and hepatomegaly due to congestive heart failure accompanying a large chorioangioma. Macintosh & Osborn (1968) described a patient with chorioangioma and acute hydramnios which reabsorbed spontaneously due to complete necrosis of the tumour, and there was no fetal congestive heart failure *in utero*.

Our patient had a large placental chorioangioma which caused fetal tachycardia, increased cardiac output, pericardial effusion and severe hydramnios. Spontaneous resorption

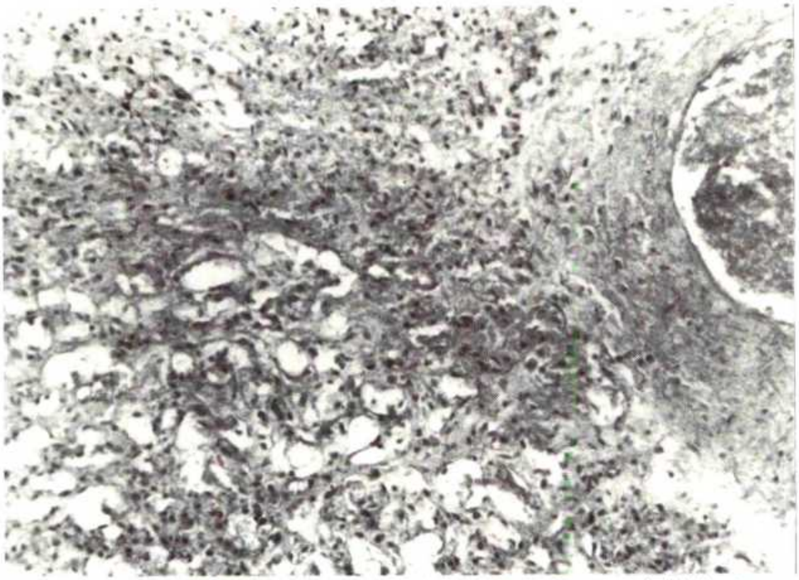

Fig. 4. A necrotic calcified chorioangioma in an otherwise normal third trimester placenta. Haematoxylin and eosin. Original magnification × 63.

of the hydramnios, following necrosis of the tumour, resulted in return of cardiac function to normal and a favourable pregnancy outcome.

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SHORT COMMUNICATIONS

Increased platelet reactivity in pregnancy-induced hypertension and uncomplicated diabetic pregnancy: an indication for anti-platelet therapy?

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Platelets are thought to play a major role in the pathogenesis of pregnancy-induced hypertension (PIH) (Howie 1977). Increased platelet reactivity may contribute to obstruction of the uteroplacental microcirculation by platelet aggregates and by release of vasoactive substances such as thromboxane A_2. Recent studies have shown that in pregnancies at high risk of PIH and growth retardation anti-platelet therapy with low-dose aspirin can prevent development of these complications (Beaufils *et al.* 1985; Wallenburg *et al.* 1986; Elder *et al* 1988). Consequently much excitement has been generated by the possibility of prevention of these disorders by anti-platelet therapy, although it is difficult to identify patients at high risk of these disorders, especially primigravidae. Pregnancies in patients with diabetes mellitus have an increased incidence of PIH, and platelet reactivity is known to be enhanced in non-pregnant diabetics, especially those with vascular disease (Halushka *et al.* 1983).

It has recently become possible to study platelet aggregation in whole blood. This is more physiological than the traditional indirect turbidometric techniques using platelet-rich plasma, as it leaves platelets in their natural milieu surrounded by red cells and white cells which can themselves influence platelet behaviour. In addition it employs a sensitive direct counting technique. There is little information on platelet reactivity in pregnancy using this technique and to our knowledge there has been no previous study assessing platelet function in diabetic pregnancy. The aim of this study was to measure platelet reactivity in whole blood in patients with PIH and uncomplicated diabetic pregnancy and to compare this with normal third trimester pregnancy and non-pregnant healthy women of similar age.

Methods and results

The following groups were studied: 12 normal healthy non-pregnant women, 14 women with uncomplicated pregnancies, 12 women with PIH, and 10 pregnant diabetic patients. The groups were comparable in terms of age and parity. Blood samples were obtained in the third trimester; mean gestation at sampling was 32 weeks (SD 3·3, range 28–37) for the normal group, 31 weeks (SD 2·9, range 28–36) in the PIH group, and 33 weeks (SD 3·0, range 28–37) in the diabetic group. No patient had taken any drugs for a minimum of 2 weeks before the venepuncture. None of the diabetic patients had a pregnancy complicated by PIH or growth retardation. PIH was defined as a mean diastolic blood pressure >90 mmHg for 24 h in patients who had been normotensive in the first trimester. Eight of the PIH group had significant proteinuria (>0·3 g/24 h) and all patients were electively delivered preterm (mean gestation at delivery 34·3 (SD 2·6) weeks). The birthweights of three of the infants in this group were <10th centile for gestation. Venous blood was obtained without stasis and anticoagulated with 3·8% trisodium citrate. Platelet aggregation studies were performed as previously described (Greer *et al.* 1987) using the Clay Adams Ultra-Flo 100 whole blood platelet counter. As platelets become activated by a stimulus such as ADP they clump together and form aggregates. The Ultra-Flo 100 only counts single platelets and therefore the platelet count will fall as aggregation occurs. The greater the sensitivity to the aggregating stimulus, the greater the fall in platelet count. In the following experiments platelet count was determined at baseline then at 1, 3 and 5 min after addition of the aggregating stimulus. At all times two platelet counts were measured and the mean recorded and expressed as a percentage of the baseline value. Final concentrations of 2 μM and 4 μM ADP were used as the aggregating stimuli. These concentrations of ADP were chosen as they caused minimal aggregation in normal pregnancies and non-pregnant patients, and the method would therefore be sensitive to any increased aggregatory response to these stimuli.

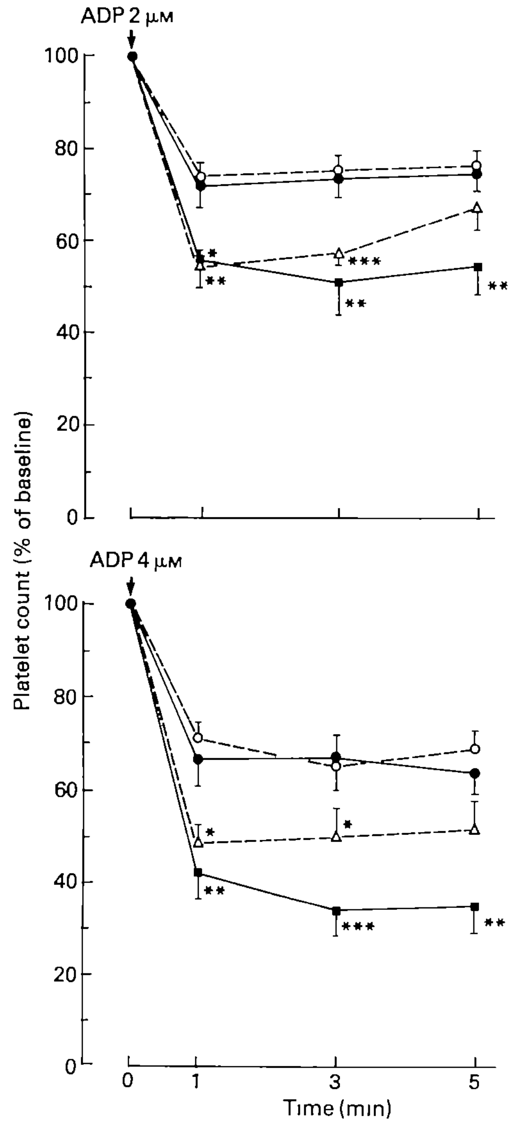

Fig. 1. ADP-induced aggregation in normal non-pregnant women (○), normal third trimester pregnancies (●), uncomplicated diabetic pregnancy (△) and PIH (■). All points are mean (± SEM) Statistical analysis by Student's t-test for unpaired data. $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

All studies were completed within 2 h of venepuncture.

Results are illustrated in Fig. 1. There was no difference in platelet reactivity between normal non-pregnant and normal pregnant patients. In the PIH group there was a significant increase in platelet aggregation at all time points studied. In the pregnant diabetic group platelet aggregation was significantly increased at 1 and 3 min but unlike the PIH group this aggregation was reversible with a reduction in aggregation noted at 5 min. These findings occurred with both concentrations of ADP used. The enhanced reactivity may be due to increased thromboxane A_2 production from platelets in patients with PIH and diabetes although the reversibility of the response in the diabetic group suggests that this may not be so marked in diabetic pregnancy.

Conclusion

These results confirm that platelet reactivity is enhanced in PIH, using the technique of whole blood platelet aggregation, and emphasizes the relevance of anti-platelet therapy in these patients. In addition the results show that platelet reactivity in whole blood is increased in uncomplicated diabetic pregnancy, a feature not previously described. Platelet aggregation is known to be enhanced in non-pregnant diabetics, especially those with vascular disease (Halushka *et al.* 1983) possibly secondary to a metabolic abnormality resulting in increased platelet thromboxane A_2 production. This enhanced reactivity can be reversed by aspirin therapy in the non-pregnant (Halushka *et al.* 1983). The increased risk of PIH in diabetic pregnancy may be related to increased platelet reactivity as described in this study. This raises the possibility that diabetic pregnancy itself may be an indication for the prophylactic use of anti-platelet therapy with low-dose aspirin, and further studies are now required to examine this hypothesis and determine whether such patients would benefit from this therapeutic strategy.

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Can risk factors be identified for failure of laser treatment to the cervix?

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Carbon dioxide laser vaporization of the cervical transformation zone is currently a popular method of treating cervical intraepithelial neoplasia (CIN). It is commonly performed as an outpatient procedure, and published success rates vary between 87% and 98% (Jordan *et al.* 1985). However, invasive disease following laser vaporization has been reported (Evans & Monaghan 1983). Various hypotheses have been advanced to explain treatment failure, but none has yet been tested. The aim of this study was to investigate factors possibly responsible for the failure of treatment.

Patients, methods and results

All patients in this study were treated in the outpatient department of the Women's Hospital, Birmingham, between 1984 and 1985. The selection of patients, technique of vaporization and the schedule of outpatient follow-up after treatment have been described previously (Jordan *et al.* 1985). Information relating to patient and disease characteristics, operative procedure and patient follow-up, were recorded on a form in routine use in the colposcopy clinic.

Laser treatment was considered to have failed when histological examination of a colposcopically directed biopsy or cone biopsy revealed CIN within 1 year of treatment (21 patients) or when repeated cytological and colposcopic examination showed abnormality to persist (3 patients).

Laser treatment was considered successful when repeated cytological and colposcopic examination detected no evidence of abnormality within 1 year of treatment.

Failures were identified by a search of registration forms for colposcopy clinic attenders between 1984 and 1985 inclusive. For each laser failure identified, the next four patients fulfilling the criteria for laser success were taken as controls. The following risk factors were studied: age, parity, grade of CIN, presence of human papillomavirus (HPV) infection (either cytological, colposcopic or histological evidence), histological evidence of HPV infection alone, seniority and experience of laser surgeon, use of local anaesthesia, depth of destruction achieved, discomfort experienced, and bleeding during treatment. Patients were not included in the study if the operator had recorded that the degree of bleeding was such as to prevent adequate completion of treatment or if the degree of discomfort experienced by the patient resulted in the procedure being abandoned.

As cases and controls were not actively matched with respect to any relevant characteristic, an unmatched analysis was appropriate. Relative risks (RR) for treatment failure were estimated by the odds ratio, and 95% confidence intervals for RR were calculated.

There were 24 cases and 96 control subjects in the study. The distribution of subjects according to the various factors, relative risks and confidence intervals are shown in Table 1. No factor was found to have a significantly elevated or reduced RR for laser failure, and exploration of subclasses of parity, grade of CIN and seniority of surgeon did not change this finding.

Discussion

The inexperience of the operator, age of the patient and the presence of HPV infection have

Table 1. Cases and controls by risk factors, relative risks (RR) and 95% confidence intervals (CI)

Factor	Failure (Cases) (*n* = 24)	Success (Controls) (*n* = 96)	RR	95% CI
Age (years)				
≥35	5	19	1·07	0·35 to 3·22
<35	19	77		
Parity				
≥3	4	13	1·28	0·38 to 4·34
<3	20	83		
Histology				
CIN III	15	46	1·81	0·72 to 4·54
Other	9	50		
Evidence of HPV infection				
Present	6	42	0·43	0·16 to 1·17
Absent	18	54		
HPV histology				
Positive	3	19	0·58	0·16 to 2·15
Negative	21	77		
Operator				
Consultant	8	28	1·21	0·47 to 3·16
Other	16	68		
Depth of destruction (mm)				
<7	2	4	2·09	0·36 to 12·15
≥7	22	92		
Local anaesthesia used				
Yes	2	5	1·66	0·30 to 9·10
No	22	91		
Discomfort noted				
Yes	17	53	1·97	0·75 to 5·19
No	7	43		
Bleeding noted				
Yes	7	48	0·41	0·16 to 1·08
No	17	48		

been reported to increase the risk of failure of laser treatment (Ali *et al.* 1986). Our point estimates do not support these opinions. They support but cannot confirm the importance of achieving an adequate depth of destruction (Anderson 1982) and the increased risk of failure associated with CIN III lesions (Wright & Davies 1981).

In this study patient discomfort during treatment was associated with a higher risk of failure. We did not observe any beneficial effect from the local anaesthetic agent which was given to 10% of patients in this series, although it was only given when the patient was already experiencing discomfort and we have no information as to the effectiveness of the analgesia obtained. It may be, therefore, that the outcome in this small group reflects the elevated risk associated with mild degrees of discomfort. We now routinely administer a local anaesthetic and vasoconstrictor agent before laser treatment. The minor degrees of bleeding reported by operators in this series did not increase the risk of laser failure.

There are two problems encountered when investigating the subject of failure of laser treatment. The first relates to the definition of failure. Previous studies have employed differing criteria: either cytological or colposcopic abnormality at follow-up (Popkin 1983; Baggish 1980), evidence of residual HPV infection alone or in combination with CIN (Goldberg *et al.* 1985), or the need to undertake further treatment other than repeat vaporization (Ali *et al.* 1986). Definition of failure based on cytology alone may be inadequate, as the false-negative rate of cytology in this situation has been reported as 33% (Maclean *et al.* 1987). Definitions of failure which rely on the presence of colposcopic abnormality alone are unsatisfactory because immature metaplastic epithelium and CIN can have similar colposcopic appearances (Cartier 1984).

We have therefore chosen a definition of treatment failure based where possible on the histological demonstration of residual CIN in 21 patients or on the presence of both cytological and colposcopic abnormality in three patients.

The second problem we have encountered relates to the sample size necessary to show clinically important and statistically significant association. This study leads us to believe that high-grade CIN lesions, inadequate depth of destruction and discomfort experienced during treatment are factors which may lead to increased risk of treatment failure. The point estimates of relative risk associated with these factors are all approximately 2·0. In order to demonstrate such a risk to be statistically significant, with exposure rates in the control population being (approximately) 50, 5 and 50% respectively, we should require a study size of 118 cases (and 4 controls per case) for the first and third factors, and 328 cases and 1312 controls for the second, at the conventional 5% level of significance with 90% power. These calculations could not be made in advance of the study for there were no published estimates of relative risk for the factors studied. If our point estimates of relative risk are indeed of the correct order of magnitude, it would require a study almost five times the size of this one to demonstrate the effect statistically.

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BLAIR BELL RESEARCH SOCIETY

Papers presented at the meeting on 6 May 1988 at the Royal College of Obstetricians and Gynaecologists

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Skeletal bone density changes in patients receiving LHRH agonists by W. H. Matta, R. W. Shaw, R. Hesp* & R. Evans*. Academic Department of Obstetrics and Gynaecology, Royal Free Hospital, London NW3 2QG, and *Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ

Luteinizing hormone-releasing hormone (LHRH) agonists, by inducing reversible hypo-oestrogenism, can be therapeutically useful in several sex hormone dependent conditions in premenopausal women. It is well established that accelerated bone loss occurs in women with oestrogen deprivation states. We studied bone density changes resulting from treatment with the LHRH agonist buserelin (Hoechst Pharmaceuticals, UK) and the longer-term changes observed 6 months after treatment and after resumption of ovarian activity in these women. Each of 13 premenopausal women received 400 μg of intranasal buserelin every 8 h for 6 months as treatment for endometriosis. Cortical bone mineral content in the right femoral mid-shaft, measured by dual photon densitometry, and trabecular bone density in the lumbar vertebrae (L 2–4), measured by quantitative computed tomography, were assessed before and at the end of 6 months' buserelin treatment and again 6 months after treatment. Mean serum oestradiol-17 β levels were significantly ($P<0{\cdot}001$) lower during the period of treatment. There was a marginally significant ($P = 0{\cdot}07$) reduction of only 0·9% in the mean femoral cortical bone mineral content at the end of treatment. Six months after the end of treatment cortical bone mineral content was not significantly different from the pre-treatment values. The significant reduction in the mean lumbar vertebral trabecular bone density of 5·9% ($P<0{\cdot}02$) at the end of treatment was regained within 6 months afterwards. The potential risk of bone loss should be a major consideration when determining the dose, duration and timing of possible repeated courses of therapy with LHRH agonists.

Screening for ovarian cancer: a multimodal approach incorporating CA 125, pelvic examination and ultrasound by I. J. Jacobs, I. Stabile, J. Bridges*, P. Kemsley*, C. Reynolds†, J. G. Grudzinskas* & D. H. Oram*. Department and *Academic Unit of Obstetrics and Gynaecology and †Department of Radiology, The London Hospital, Whitechapel, London E1

Postmenopausal women over 45 years of age were recruited for an ovarian cancer screening programme incorporating serum CA 125 measurement and vaginal examination as initial tests, and real-time ultrasonography as a secondary procedure in selected women. This report describes the results of the first 1010 women screened. A stage Ia clear-cell carcinoma of the ovary was detected in one patient who had a positive CA 125 of 32 U/ml, pelvic examination and ultrasound scan. No other ovarian cancers had developed in the remaining 1009 volunteers at 1-year follow-up. The normal range for serum CA 125 in postmenopausal women was established; the 90th, 95th and 99th centiles were 23·2 U/ml, 26·3 U/ml and 36·8 U/ml respectively. The specificity for ovarian cancer of serum CA 125 measurement (upper limit 30 U/ml) and vaginal examination individually were 97·0% and 97·3% respectively. The combinations of serum CA 125 with ultrasound and vaginal examination with ultrasound achieved specificities of 99·8% and 99% respectively. A specificity of 100% was achieved by serum CA 125 measurement with vaginal examination and the combination of all three tests. We conclude that the specificity of serum CA 125 measurement, with either vaginal examination or ultrasound, is sufficient to warrant further investigation of the sensitivity of these combinations of tests for early-stage ovarian cancer.

The effects of anorexia nervosa on skin thickness, skin collagen content and bone density by M. Savvas, J. Treasure*, M. Brincat, C. Moniz, I. Fogelman† & J. Studd. Dulwich Hospital, *Institute of Psychiatry and †Guy's Hospital, London

A cross-sectional study of skin thickness, skin collagen content and bone mass was performed in a group of 36 women with anorexia nervosa and 33 normally menstruating women. Skin thickness was determined radiologically and collagen content estimated from punch biopsies taken from the lateral aspect of the thigh. Bone density was estimated using dual photon absorptiometry. Median skin thickness in the control group was 0·88 mm compared with 0·70 mm in the anorectic women ($P<0{\cdot}01$). Median skin collagen content was 209 μg/mm² and 164 μg/mm² in the two groups respectively ($P<0{\cdot}05$). Median bone density in the control group was 0·93 gHA/cm² and 0·84 gHA/cm² at the lumbar spine and neck of femur respectively; this was significantly lower at both sites in the anorectic women with values of 0·77 gHA/cm² and 0·65 gHA/cm² at the spine and femur. We have shown previously that following the menopause there is a reduction in skin thickness and skin collagen content. This study demonstrates that anorexia nervosa has similar effects on skin and bone and suggests that hypogonadism may be a common mechanism.

Psychological assessment of hirsute women by J. E. Dixon, B. H. Hicks* & M. G. Chapman. Departments of Obstetrics and Gynaecology, and *Endocrinology, Guy's Hospital, London

In the general assessment of the hirsute patient the psychological effect of hirsutism is important and a common reason for referral. The clinical impression has been that these women are more depressed and neurotic than other regular clinic attenders and many of them reported worsening mood changes while taking anti-androgen medication. To investigate this, 48 hirsute women have been studied over the last 18 months by psychiatric assessment using the Beck Depression Inventory (BDI) and the 60-question General Health Questionnaire (GHQ). Previous studies of large random samples of women have shown that scores of >12 in both the BDI and GHQ are associated with increased psychiatric morbidity. In a random sample of women 20–25% will fall in this range. In the hirsute population studied 66% had GHQ scores >12 and 45% had BDI scores >12 before medical treatment. Patients were prescribed either cyproterone acetate (50 mg daily on days 5–15) with ethinyl oestradiol (30 μg daily on days 5–25) or spironolactone (50 mg twice daily) with Ovysmen daily for 21 days. After 6 months they were changed to the alternative treatment without a break. To date 18 patients have completed the trial and there was a significant improvement in their psychological state as well as their physical appearance. Mean GHQ scores dropped from 13·8 to 8·2 ($P<0{\cdot}05$ Wilcoxon signed rank); 24 patients failed to complete 1 year of treatment, 12 of whom gave mood changes as the reason for failure. This could not be predicted from the BDI or GHQ scores before treatment. In conclusion these women were a depressive group and successful treatment of their hirsutism significantly improved their mental state.

The immunosuppressive effects of human cervical mucus by A. G. Pockley, C. L. R. Barratt* & A. E. Bolton. Department of Biological Sciences, Sheffield City Polytechnic and *University Department of Obstetrics and Gynaecology, Jessop Hospital for Women, Sheffield

The female reproductive tract is not an immunologically privileged site. However, inseminated sperm do not provoke an immune response in normal women, although sperm may be immunogenic by normal criteria. Seminal plasma contains a high level of immunosuppressive activity, which may prevent a response against sperm in the vagina by preventing the direct interaction of sperm antigens with the female immunocompetent cells and/or interfering with their responsive capacity. Nevertheless, sperm may present an immunological challenge during passage through the cervical canal. We investigated the effects of cervical mucus on one in-vitro measure of cell-mediated immunity, lymphocyte responsiveness to the mitogen phytohaemagglutinin (PHA). Cervical mucus was obtained from normal women and extracted in phosphate-buffered saline by ultrasonication. The insoluble material was removed by centrifugation and the extracts assayed for total protein concentration. Pooled extracts significantly suppressed the proliferation of PHA-stimulated peripheral blood lymphocytes by 66% compared with control cultures in a dose-dependent manner. On the incorporation of eight cervical mucus extracts into the PHA assay there was no significant correlation between wet weight of sample extracted or soluble protein content with the suppressive activity. Such activity was not removed by dialysis of the extracts, indicating the involvement of high molecular weight factors. These findings indicate the presence of an immunosuppressive factor in cervical mucus which could function to protect inseminated sperm during passage through the cervix.

Prostaglandin E_2 and $F_{2\alpha}$ in follicular fluid from patients undergoing in-vitro fertilization (IVF) by E. M. Smith, F. Anthony, S. C. Gadd & G. M. Masson. Department of Human Reproduction and Obstetrics, University of Southampton, Princess Anne Hospital, Southampton

Rising levels of prostanoids in pre-ovulatory follicular fluid support a role for their acting at follicular level in the process of oocyte maturation. We examined the relation between prostanoid levels in follicular fluid and the quality of the matched oocyte assessed by its IVF outcome. Overall, 219 follicular fluids and oocytes were obtained from 32 patients undergoing hyperstimulation on our IVF programme. Follicular maturation was induced with intranasal buserelin (Hoechst Pharmaceuticals, UK) and intramuscular human menopausal gonadotrophin. Follicular aspiration was performed 34 h after hCG administration at optimal follicular size as designated by ultrasound. Concentrations of PGE_2 and $PGF_{2\alpha}$, oestradiol and progesterone in follicular fluids were measured by radioimmunoassay. Follicular PGE_2 and $PGF_{2\alpha}$ levels for oocytes were allocated to data groups dependent on fertilization of the respective oocyte and occurrence of pregnancy. Results showed that an optimal range existed for the prostaglandin ratio in follicular fluid for the pregnant group which was statistically different from the unfertilized group and the fertilized group that did not become pregnant ($P<0{\cdot}01$). There was a highly significant correlation between the PGE_2 and $PGF_{2\alpha}$ concentrations ($P<0{\cdot}001$) and significant correlations between PG and oestradiol concentrations ($P<0{\cdot}01$)

The influence of raising maternal blood pressure by infusion of angiotensin II on maternal carotid artery, uterine and umbilical artery blood flow velocity in early pregnancy by Ph.Loquet, F. Broughton Pipkin*, E. M. Symonds* & P. C. Rubin. Departments of Therapeutics and *Obstetrics, University Hospital, Nottingham

The recent developments in Doppler ultrasound allow us to look at changes in blood flow velocity. To study the reaction of fetal and maternal haemodynamics to a rise of maternal blood pressure, we infused angiotensin II at increasing rates of 4, 8, 16 or more ng/kg/min in 26 patients (10 non-pregnant, 8 first- and 8 second-trimester pregnant women), until a diastolic blood pressure rise between 20 and 30 mmHg was obtained. Recordings of maternal carotid artery, arcuate and umbilical artery showed that both in the first and second trimester there was a significant ($P<0{\cdot}01$) increase in umbilical artery pulsatility index (PI) (12% and 11% respectively) for a mean diastolic blood pressure increase of 35% at the 32 ng/kg/min dose. A significant linear correlation was found between blood pressure changes and umbilical artery PI. The mean PI in the arcuate artery decreased with increasing blood pressure although this was not statistically significant because of the large SDs. A highly significant decrease in maximal velocity was seen from a dose of 8 ng/kg/min in the non-pregnant group and from 16 ng/kg/min in the pregnant group. There was a strong linear correlation ($r>0{\cdot}7$) between blood pressure and maximal velocities. Again PI decreased significantly with increasing blood pressure. This was unexpected because angiotensin is a vasoconstrictor. Our first results show that it is possible to influence the fetal circulation directly through maternal blood pressure control. Even if there is some evidence from umbilical artery studies that PI is related to resistance, its significance in other vessels might be questioned from these data.

Amniotomy does not increase plasma oxytocin in late pregnancy by S. Thornton, J. M. Davison & P. H. Baylis*. Department of Obstetrics and Gynaecology, Princess Mary Maternity Hospital, and *Endocrine Unit, Royal Victoria Infirmary, Newcastle-upon-Tyne

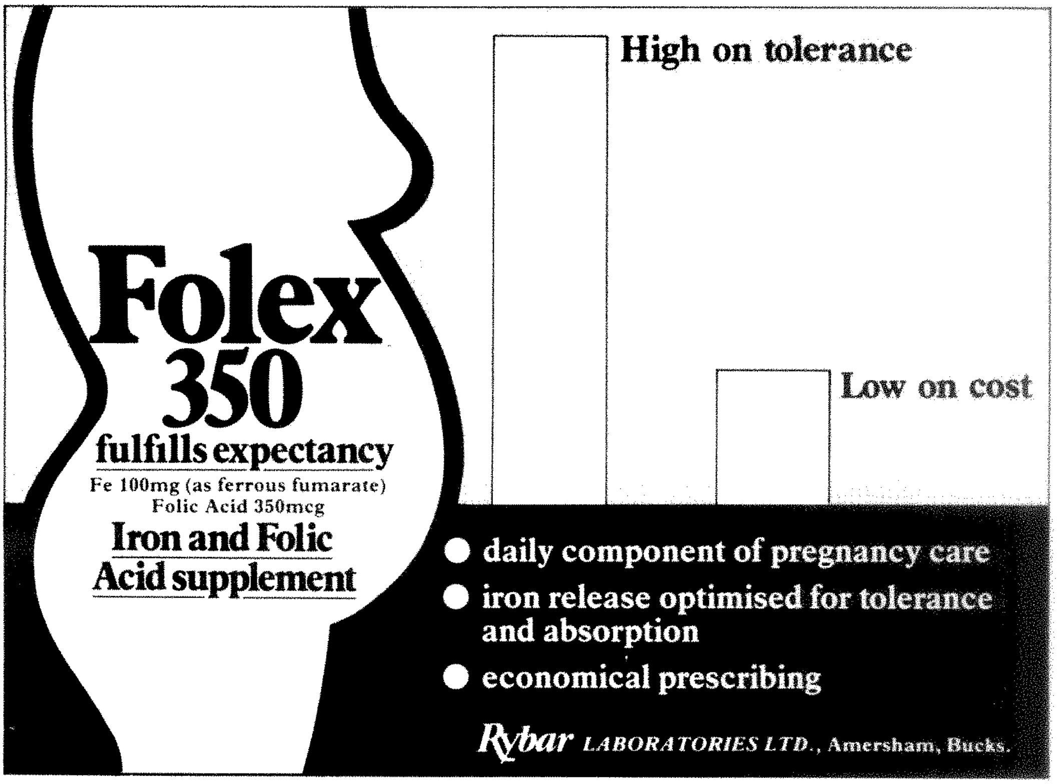
Amniotomy can initiate labour in late pregnancy. Oxytocin may mediate the uterine response via the Ferguson reflex, whereby vaginal or cervical manipulation causes an increase in the plasma oxytocin concentration. Using a new sensitive method to measure plasma oxytocin which incorporates a technique that completely inhibits oxytocinase activity, we determined plasma oxytocin profiles before, during and after amniotomy in 16 randomly allocated patients admitted for induction of labour at term. Venous blood samples were taken at 1-min intervals for 30 min. Eight patients had an amniotomy 10 min after the start of sampling (time taken for procedure 2–7 min) and 8 patients (control) were not induced until the end of the sampling period. All sample syringes contained oxytocinase inhibitors, the plasma was extracted by the Florisil method within 1 h and oxytocin was measured by solid-phase radioimmunoassay within 2 weeks. Plasma oxytocin levels did not fluctuate during sampling, allowing the mean concentration to be determined for each patient before, during and after amniotomy. The mean plasma oxytocin level was 1·6 (SE 0·5) pmol/l before, 1·6 (SE 0·5) pmol/l during ($P = 0·3$; Student's paired *t*-test; mean difference 0·05 pmol/l) and 1·7 (SE 0·5) pmol/l after amniotomy ($P = 0·2$; paired *t*-test, before *vs* after; mean difference 0·1 pmol/l). The mean plasma oxytocin level in control patients was 1·0 (SE 0·1) pmol/l and was not significantly different ($P > 0·1$; Student's unpaired *t*-test) at any time from that in patients undergoing amniotomy. We conclude that amniotomy is not associated with a rise in plasma oxytocin either during or immediately after the procedure. The results suggest that the Ferguson reflex does not operate during vaginal or cervical manipulation in late human pregnancy.

Interaction of corticotrophin-releasing factor (CRF) and oxytocin in superfused pregnant myometrium by H. W. P. Quartero, C. D. A. Wolfe, P. Carabelli, C. H. Fry* & M. T. Jones. Departments of Gynaecology and *Physiology, UMDS, St Thomas's Campus, London SE1

We have reported elevated levels of CRF in the third trimester with significantly higher levels in pregnancy-induced hypertension and up to 10 weeks before idiopathic preterm labour As a synergistic effect between CRF and oxytocin/vasopressin has been described at pituitary level we have tested this model in human myometrium obtained from caesarean sections at term. Small strips (10 mg) of myometrium were mounted in an organ bath and superfused with a tyrodes solution at a rate of 10 ml/min. A regular contraction pattern was induced by field stimulation with tetanic pulses of 10 ms, 4 Hz for 4 s every 2 min. Superfusion with test solutions was maintained for 20 min with a 30-min control period between stimulations. Response was expressed as a proportionate change from the 15-min control period before stimulation. CRF^{-10}M before any superfusion with oxytocin showed a negative inotropic effect, while this effect was reversed to a positive inotropic effect after priming with oxytocin and CRF combined ($P < 0·005$). The positive inotropic effect of CRF was equivalent to that of oxytocin^{-9}M before any exposure to CRF. The positive intropic effect of oxytocin was markedly enhanced by priming with CRF and oxytocin ($n = 3$). Whether there is addition or potentiation remains to be shown. This preliminary report of the inotropic effects of CRF in human myometrium and of its interaction with oxytocin outside the hypothalamo-pituitary axis would imply that CRF might reduce uterine activity before labour, but accelerate labour once it has started.

Needle electromyography (EMG) of the urethral skeletal sphincter; denervation injury and incontinence by M. S. Krieger & S. L. Stanton. Department of Obstetrics and Gynaecology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE

Electrophysiological studies of the anal sphincter and lateral pelvic floor have suggested that denervation injury, secondary to childbirth, is a factor in the development of incontinence. We performed EMG using concentric (CN) and single-fibre (SF) needles on the urethral sphincters of three groups of premenopausal women who had not had previous bladder neck surgery: nulliparous controls (CN–6, SF–5), continent multiparas (CN–10, SF–4) and stress incontinent multiparas (CN–23, SF–13). Using the concentric needle technique ($n = 39$) there were significant differences between the groups in mean motor unit potential durations (MUPD). For nulliparas *vs* parous controls (correcting for age), $t = 4·99$ ($P < 0·001$) and parous continent *vs* incontinent, $t = 3·18$ ($0·001 < P < 0·01$). Previous hysterectomy did not influence mean MUPD. With the SF method ($n = 22$) there were significant differences in fibre density between nulliparous and parous continent ($z = 2·79$, $P = 0·005$) and parous continent and incontinent patients ($z = 2·69$, $P = 0·006$). MUPD's measured by SF needle in these 22 patients were compared. There was a significant difference between the two continent groups ($t = 5·84$, $P > 0·001$), but not between the two parous groups ($t = 1·49$, $0·1 < P < 0·2$). These differences indicate that the urethral sphincter is partially denervated in pregnancy and/or childbirth, and this appears to be more marked in patients who have clinical stress incontinence than in those who do not. SFEMG appears to be a more sensitive tool than MUPD analysis for detecting differences between these clinical groups.



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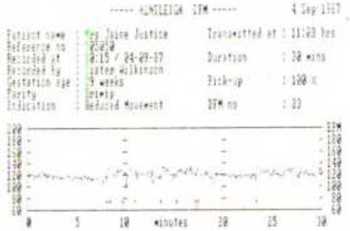

----- HUNTLEIGH DFM ----- 4 Sep 1987

Patient name : Mrs Jane Justice
Reference no : 02020
Recorded at : 8:15 / 04-09-87
Recorded by : Sister Wilkinson
Gestation age : 39 weeks
Parity : Primip
Indication : Reduced Movement

Transmitted at : 11:03 hrs
Duration : 30 mins
Pick-up : 100 %
DFM no : 33

0
5
10
minutes
20
25
30

Contact : Mrs Justice (home) 0222 485885 Action : Repeat FM

Seen by : Dr J B Watton At : 11:10 / 04-09-87

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British Journal of Obstetrics and Gynaecology
November 1988, Vol. 95, p. 1213

CORRESPONDENCE

The effect of pelvic floor exercises in the treatment of genuine urinary stress incontinence in women at two hospitals

Dear Sir,
We read with interest the paper by Henalla *et al.* [*Br J Obstet Gynaecol* (1988) **95**, 602–606] on pelvic floor exercises.

The result of this work indicates the value of a specialized physiotherapy regimen which obviates the need for surgery in a significant proportion of women who comply with the treatment programme. The paper stresses the need for a specialist physiotherapist with the ability to perform vaginal examinations, and the success of the project undoubtedly rests on the close co-operation of the two physiotherapists involved.

It is therefore of concern to note that the authors of this paper are four gynaecologists and a professor of geriatrics and that the physiotherapists are not included as co-authors. For whatever reason they are omitted, this is most unfortunate. Paramedical professions need to be involved in research-based work for the recognition and development of their professional skills and practices. Where different disciplines are equally interdependent in a research project it cannot be correct that at the conclusion of the work only the names of the doctors appear as authors.

Physiotherapists qualifying with a chartered diploma or a degree follow a strongly academic as well as practical training and subsequent specialization into areas of professional practice requiring postgraduate training is common. The specialist physiotherapists for this project would have been involved in postgraduate work in order to carry out their treatments. Physiotherapists are not technicians but practitioners in their own right.

These comments apply to the role not only of the physiotherapist but to the roles of all paramedical professions and, in the case of obstetrics, notably to the role of the midwife. Both the medical profession and the paramedicals are at fault in not insisting on multidisciplinary authorship as an indication of their mutual dependence on each other for expertise in a research project of this nature.

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Reference

Henalla, S. M., Kirwan, P., Castleden, C. M., Hutchins, C. J. & Breeson, A. J. (1988) The effect of pelvic floor exercises in the treatment of genuine urinary stress incontinence in women at two hospitals. *Br J Obstet Gynaecol* **95**, 602-606.

Authors' reply

Dear Sir,
We acknowledge Mr and Mrs Hare's comments and thank them for their interest.

There is no doubt that the paramedical professions such as physiotherapists play a very important role. I have indeed acknowledged the work of both physiotherapists involved in the study. However, I feel that I have done most of the physiotherapy by myself at the Lincoln County Hospital. As you understand from the paper, the second group of patients that were treated at Leicester General Hospital were part of a large controlled trial that was organized in conjunction with our physiotherapist, Mrs Robinson. It is intended to publish this work with Mrs Robinson as a co-author.

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British Journal of Obstetrics and Gynaecology
November 1988, Vol. 95, pp 1214–1216

BOOK REVIEWS

Doppler Ultrasound Measurement of Maternal–Fetal Hemodynamics (Reproductive and Perinatal Medicine VIII) Proceedings of a National Institute of Child Health Development Research Planning Workshop held in Washington DC 1986 edited by D. Maulik & D. McNellis. Perinatology Press, Ithaca, New York, 1987, 306 pages with figures and tables, $60.00

The use of Doppler ultrasound to assess uteroplacental and fetoplacental blood flow is of considerable current interest. Whether or not this technology is yet ready to guide clinical obstetric management is a matter for debate. This volume brings together the contributions of a multidisciplinary group which met during September 1986 under the patronage of the American National Institute of Child Health and Human Development. Physical principles are discussed as are available clinical studies and lessons learned from the use of Doppler ultrasound in other branches of medical practice. There are excellent accounts of the physiology of maternal and fetal haemodynamics and of the use of animal models to investigate the pathophysiology of pregnancy. Participants at the workshop reached agreement on a number of recommendations for future research which are of relevance to physicists, obstetricians, fetal physiologists and others. They stress (correctly in my opinion) the need for randomized trial before clinical application. Such compilations are often of uneven quality—this one is well written throughout. It should be read by all who are interested in this new field of pregnancy assessment.

J. P. Neilson

Neonatal Cardiopulmonary Distress edited by George C. Emmanouilides & Barry G. Baylen. Year Book Medical Publishers, Inc., Chicago, 325 pages with figures and tables, £37.50

Congenital heart disease and respiratory diseases of the neonatal period tend to be considered separately in textbooks and by the specialists dealing with them. This compact yet detailed book effectively shows the important links between these two areas in both symptomatic and physiological terms. The authors point out the importance of the new technologies of Doppler and two-dimensional echocardiography which have enabled easier non-invasive structural and functional data to be acquired to aid diagnosis and management of these conditions. The common role of pulmonary hypertension and ductal patency in many apparently unrelated cardiorespiratory diseases is emphasized.

The 12 chapters cover fetal and neonatal cardiorespiratory physiology, resuscitation, congenital cardiac and lung disorders, the effects of persistent pulmonary hypertension, neonatal respiratory disease, and related disorders and complications of management.

The six authors are all professors of cardiology or neonatology in Los Angeles, and Professors Emmanouilides and Baylen have obviously been able to make them produce their contributions in a short time so that the resulting book is crisply up to date. There is also a marked uniformity of style throughout, giving the impression of a single-author book.

Criticisms include the lack of illustrations of two-dimensional echocardiography and Doppler studies and the curious handling of references. The text is in a very small typeface, and each chapter has a long list of references at the end in a larger typeface, numbered and in alphabetical order. No reference is made to them at all in the text, however, rendering them fairly useless and marring an otherwise impressive production. At the level at which this book is intended to be used, one would expect the reader to be interested in the source of the author's data and opinions.

Richard Cooke

Cytological Screening in the Control of Cancer: Technical Guidelines World Health Organization, Geneva, 1988, 52 pages with figures and tables, Sw.Fr.11

This handbook appears, primarily, to be aimed at setting up screening programmes for the prevention of cervical cancer in underdeveloped countries.

The first chapter is well written, succinct and gives an understanding of the background and objectives of any screening programme. It highlights the wide variation in the number of cases occurring in different countries but unless related to population this table is unhelpful. The cost of the programme is largely dependent on the number of smears each technician reads in a day. The authors expect this to be 80–90 slides per day and remark that this drops to 20–25 slides if *Chlamydia*, trichomoniasis and *Monilia* are to be diagnosed. This is difficult to understand as these should be incidental findings. The objective of screening is to look for cell changes suggestive of malignancy.

The nomenclature used for reporting abnormal smears is given as degrees of dysplasia, which is a histological not a cytological diagnosis. This is given in both chapters 2 and 3, and a defect in the book is this repetition between various chapters making the book difficult to read through. Points such as the need to appoint the designated person in overall control of all aspects—collection, laboratory, reporting, and recall—are missed.

Chapter 8, on equipment and supplies, taken in conjunction with Annex 1 and 2 will be helpful. Prices are based on US dollars (1986). The further reading suggested is comprehensive and adequate.

This book can be recommended for those setting up screening programmes but it tends to make the whole exercise more complicated than it need be.

J. Elizabeth Macgregor

Osteoporosis 1987. Volumes 1 & 2. Proceedings of the Symposium on Osteoporosis, Denmark, September/October 1987 edited by C. Christiansen, J. S. Johansen & B. J. Riis. International Symposium on Osteoporosis, 1987, Vol. 1: pp.5–637; Vol. 2: pp. 641–1285 with figures and tables, D Kr. 1200

This pair of volumes reports the proceedings of the second Symposium on Osteoporosis organized by Claus Christiansen and his colleagues. The first symposium took place in 1984 and was attended by some 400 delegates. The second conference was nearly twice as big and these proceedings reflect the vast amount of data, much of it new, presented at that meeting

As with many conferences these days, the authors were instructed to bring their manuscripts with them. This precluded any editing or refereeing of the contributions. Furthermore, the size of the conference and the increased amount of work presented meant that it was no longer possible to print the discussion after the end of each paper, so that the reader has to make up his or her own mind what to believe.

As a source book for what is going on in osteoporosis research, this provides an excellent read. The accent is on clinical research rather more than on basic science studies, so some basic science research groups from the other side of the Atlantic were not represented. However, this potential defect is in many places compensated for by good review articles by well-known authors who are fully conversant with the current status of basic bone research.

Each section of the book deals with a fairly broad range of topics. The first concerns epidemiology, the second cellular mechanisms of bone remodelling, the third discusses risk fractures and the question of whether bone quality is an important determinant (with bone quantity) of fracture, and the fourth section concerns the prevention of postmenopausal osteoporosis. The emphasis then shifts on to new biochemical markers for bone turnover, cellular mechanisms of bone remodelling (a second session is devoted to this), treatment of postmenopausal osteoporosis, and finally a session on steroid-induced osteoporosis. The proceedings of mini-symposia on oestradiol, diphosphonates, calcitonin and nandrolone therapy are also included.

In the main part of the proceedings, each section is dominated by invited contributions which frequently take the form of mini-reviews. To those not deeply immersed in the field or who are approaching an aspect of bone research which is not fully familiar to them, these are the important parts of the book. But the work presented as posters generally is included in two- or three-page mini-papers at the end of each section, and this gives just enough room to provide the reader with a reasonable grasp in most cases of what the authors have attempted. In no sense are many of these contributions proper scientific publications; they do not give a guide as to how the reader could try to reproduce the results, and should be read through a critical pair of spectacles.

In conclusion, this is a useful and topical guide. Much of the work contained within it will be republished in more definitive form in the next 2 to 3 years. It is not a book for the uncritical; but it is useful for the reader interested in broadening his appreciation of the range of clinical research activity in the field of osteoporosis at the present time.

Jonathan Reeve

Books received some of which may be reviewed at a later date

Gynecological Decision Making Second edition edited by E. A. Friedman, M. Borten & D. S. Chapin. B. C. Decker, Inc., Toronto, Philadelphia, 1988, 356 pages with figures and tables, £39.50

Controversies in Breast Disease: Diagnosis and Management edited by Sharon Grundfest-Broniatowski & Caldwell B. Esselstyn. Jr. Marcel Dekker, Inc., New York, Basel, 1983, 536 pages illustrated, $180.00 ($150.00 USA & Canada)

Drug Misuse: A Practical Handbook for GPs by A. Banks & T. A N. Waller. Blackwell Scientific Publications, Oxford, 1988, 344 pages, £12.95

Gynecologic Surgery edited by Luis E. Sanz. Medical Economic Books, Oradell, New Jersey, 1988, 400 pages with figures and tables, $57.95

The Vulva edited by C. M. Ridley. Churchill Livingstone, Edinburgh, 1988, 363 pages illustrated, £60.00

Proceedings and Symposia

The Early Days of Pregnancy (Journal of Reproduction and Fertility Supplement 36) Proceedings of a Symposium held as a Valedictory Meeting for Professor Arnold Klopper at Aberdeen in September 1987 edited by A. A. Templeton & Barbara J. Weir. Available from Biochemical Society Book Depot, PO Box 32, Commerce Way, Colchester CO2 8HP, UK, £28.00

Research in Human Reproduction Biennial Report 1986–1987 WHO Special Programme of Research, Development and Research Training in Human Reproduction edited by E. Diczfalusy, P. D. Griffin & J. Khanna. World Health Organization, Geneva, 1988, 346 pages illustrated, Sw. Fr. 25.00

Directions to contributors

All papers should be sent to The Editor, *British Journal of Obstetrics and Gynaecology*, 27 Sussex Place, Regent's Park, London NW1 4RG. Papers received are assumed to have been submitted exclusively to the *British Journal of Obstetrics and Gynaecology* and *all* authors must give signed consent to publication in a letter sent with the manuscript The Editor will decide on the time of publication and retains the usual right to modify the style and length of a contribution, major changes will be agreed with the author(s)

Presentation of manuscripts

Two copies of the manuscript and illustrations should be sent. Manuscripts should be typed double spaced on one side of the paper, with wide margins. The names of author(s) and their appointment(s) and addresses at the time of preparation of the manuscript should be given Authors are advised to keep a copy of all manuscripts, as no responsibility can be accepted for loss.

Reports of original work should usually be arranged in the conventional order of summary, introduction, methods, results, discussion, acknowledgments and references, with suitable headings for each part. Two further sub-divisions, with appropriately less significant headings, can be used, the third order heading runs on with text at a paragraph opening Results may be presented in the text, in tables and/or figures, but the text should, in general, comment on rather than repeat information in tables. The summary should contain a factual account of the methods and results in not more than 150 words, rather than a description of the subject of the paper and the opinions of the author(s)

Case reports and short communications do not require a summary or introduction. Short communications should not exceed 600 words and contain no more than one table or figure.

Tables and figures

Tables and figures should be separate from the text, with their positions indicated in the text margin Both are numbered with arabic numerals. Colour illustrations are allowable only in special circumstances and the author will be expected to meet the cost of reproduction

Figures should be marked on the back (light in pencil) with the name(s) of the author(s), the figure number, and the top indicated. Each should have a descriptive legend typed on a sheet separate from the figure Photographs should be unmounted glossy bromide prints of good contrast

Figures consist of all material which cannot be set in type, such as photographs, line drawings, etc Tables are not included in this classification and should not be submitted as photographs. Original X-ray films should not be sent—glossy prints must be submitted

Line drawings should be on white board, arranged to conserve space, and should allow for reduction to 65 mm (single column) or 134 mm (double column)

Typewritten lettering should not be used on illustrations and explanations should appear in the legend not on the figure itself

The identity of patients in illustrations should be concealed by masking the face or eyes; or written permission for publication from the patient or the parent or guardian must be supplied

Units and abbreviations

Measurements should be expressed in SI units with the exception of haemoglobin (g/dl) and blood pressure (mmHg) If the original observations were recorded in other units this should be stated, together with the appropriate conversion factors. Standard abbreviations, without punctuation, are used. *Units, Symbols and Abbreviations* (1977) published by the Royal Society of Medicine provides a useful guide. Abbreviations are used sparingly and only if a lengthy name or expression is repeated frequently throughout the manuscript. Words must appear in full on first appearance in both summary and text, followed by the abbreviation in parenthesis

Drugs are described by their official names but trade names should be indicated in brackets the first time a drug is quoted in the main text

Statistics

Authors should describe clearly the design of their investigation and the source of the data All statistical methods should be specified so that it is clear which was used where Papers omitting this information may be returned without review. The basic principle to follow is to supply sufficient information about the design and methods of analysis to allow the research to be repeated by someone else

Some points of detail to note are

(1) It is not acceptable to present the results of a statistical analysis solely by a *P* value—actual results such as means or proportions should always be given, along with the test statistic.
(2) The use of confidence intervals is encouraged
(3) The symbol ± should not be used when presenting SE or SD. Presentation such as 'the mean was 14·2 (SD 3·2) mg' is preferred
(4) When paired comparisons are made it is desirable to indicate the variability of within-pair differences.
(5) Unusual methods of analysis should be described and/or referenced

Authors are referred to the general statistical guidelines of Altman *et al.* (1983) for advice about the presentation and interpretation of the statistical aspects of their research

Altman, D G., Gore, S M., Gardner, M J. & Pocock, S. J. (1983) Statistical guidelines for contributors to medical journals. *Br Med J* **286,** 1489–1493

Human experiments

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References

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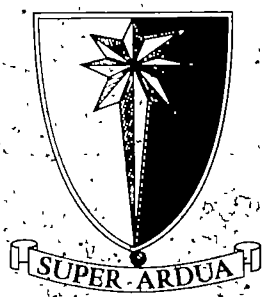

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COMMENTARY

Planned cycles for IVF and GIFT

In the 10 years since the first birth following in-vitro fertilization and embryo transfer (IVF/ET) the potential for treatment of infertility has changed dramatically. Many thousands of patients with tubal and other causes of infertility have been treated with this technique, but efficient in-vitro fertilization remains an expensive procedure requiring highly trained personnel, specialized laboratory equipment, intensive hormonal and ultrasound monitoring and a 7-day laboratory and clinical service. These factors, in conjunction with a low mean success rate of 11% (VLA 1987) and the perception that it is expensive, have led to the treatment being conducted in highly specialized units and almost exclusively on a private basis or in university departments heavily subsidized by the private sector.

Assisted conception for women with normal fallopian tubes was simplified when Asch *et al.* (1985) reported pregnancies following laparoscopic gamete intrafallopian transfer (GIFT). The increased utilization of this technique in smaller centres represented a substantial advance in the treatment of non-tubal infertility. This one-step procedure is simpler than IVF/ET as the oocytes and sperm are mixed immediately and transferred back to the tubes minimizing the need for expensive laboratory facilities. GIFT is also, in selected patients, more successful than IVF with an overall success rate of 30% (Asch *et al.* 1985).

The success and relative simplicity of the GIFT procedure has encouraged more gynaecological departments within the National Health Service (NHS) to try to adopt such a technique as the procedure of oocyte collection and tubal catheterization at laparoscopy is relatively easy to learn. The major difficulty that prevents such a successful procedure from being widely available within the facilities of the NHS is that operative procedure cannot be timed to the convenience of the surgical team, thus the daily availability of an operating theatre is required.

To obviate this problem many authors have attempted to manipulate the menstrual cycle in order to fix the date of oocyte retrieval and in this issue of the Journal (p. 1220) Bates *et al.* have reported the use of progestogens for programmed cycles of GIFT.

Wardle *et al.* (1986) first described the use of progestogens in the pretreatment cycle in a clinical IVF programme. A withdrawal bleed could be expected between 3 and 5 days after stopping progestogen. The patient is then stimulated with a standard drug regimen, normally using clomiphene citrate and human menopausal gonadotrophins (hMG) followed by human chorionic gonadotrophins (hCG) administered 2 days preceding the fixed operation day which is normally day 12 or 13 of the cycle. There are many modifications of this approach which seems to be effective (Templeton *et al.* 1984; Frydman *et al.* 1986).

However, the planning of cycles by progestogens still has problems. The first is that menstruation may not start within the expected time although this was not believed to be any disadvantage in the study by Bates *et al.* (1988). Another disadvantage is that ovulation induction with a combination of clomiphene citrate and exogenous gonadotrophins elevates the LH/FSH ratio (Jeffcoate 1984), and requires monitoring of urinary LH and oestradiols as premature luteinization can occur in 30% of women and premature ovulation occurs in 15% of cycles leading to cancellation of the planned oocyte collection (Steptoe *et al.* 1986). The elevated LH is also associated with the occurrence of postmature oocytes, poor fertilization rates and poor embryonic development which ultimately is responsible for the reduction in pregnancy rates (Stanger & Yovich 1985; Howles *et al.* 1986; Punnonen *et al.* 1988).

A more recent development for planned cycles is the use of an LHRH analogue which desensitizes pituitary function and reduces the level of endogenous gonadotrophins. When administered in conjunction with exogenous gonadotrophins it prevents premature luteinization (Fleming *et al.* 1985; Fleming & Coutts 1986), and pre-operative follicular rupture should never occur if the operation is planned within 32–36 h after hCG administration. Zorn *et al.* (1987) described programming of the cycle using norethisterone followed by an LHRH analogue and hMG which helped to prevent premature luteinization. Other workers have shown that an LHRH analogue and hMG result in a significantly higher number of oocytes collected with an increase in the pregnancy rate and a decrease in early pregnancy loss when compared with clomiphene citrate and hMG (Abdalla *et al.* 1988).

Rutherford *et al.* (1988) have also shown that such a combination results in a higher cleavage rate and better quality embryos. Both groups have shown that the use of the analogue abolished pre-operative cancellation due to ovulation. Studd *et al.* (1988) have shown that oocyte collection can be delayed in patients receiving the LHRH analogue, even if the follicles have reached the standard requirement for the administration of hCG, provided that the patient continues to receive the LHRH analogue and gonadotrophins for follicular stimulation and the hCG is administered 32–36 h before the day of the operation. They have shown that such a delay does not influence the outcome adversely as the group in whom oocyte collection was delayed showed a significant rise in the number of fertilized oocytes and a slight rise in the pregnancy rate.

Two different regimens have been recommended for ovulation induction using an LHRH analogue and gonadotrophins. The long protocol described by Porter *et al.* (1984) uses the analogue for 15 days to desensitize the pituitary; when the gonadotrophins are totally suppressed exogenous gonadotrophins are administered. This approach, though effective, is costly. A cheaper approach is the short protocol whereby advantage is taken of the initial agonistic phase of the LHRH analogue to allow greater follicular recruitment. Exogenous gonadotrophins are then administered simultaneously and the continued administration of the analogue results in suppression of endogenous gonadotrophins. This LH suppression takes 4–5 days in women with normal LH levels (Fleming *et al.* 1982) and 9–14 days in patients with initial high plasma LH levels (Fleming *et al.* 1985). Since the critical time for the damaging effects of a high LH level is near ovulation at the time of maximum follicular development, the use of the short protocol is adequate even in patients with polycystic ovaries and high LH levels. The short protocol requires fewer ampoules of gonadotrophins to stimulate the follicular development and in both the long and short protocols the timing of the operative procedure can be adjusted to the surgical teams' availability using two or four operative procedures each week, depending on the number of patients going through any particular programme.

The use of the LHRH analogue represents a considerable advance not only in the timing of the operative procedure but also in the cost-effectiveness as there is no need for LH or perhaps even oestradiol monitoring and there are no pre-operative cancellations due to premature ovulation. Apart from being simpler this combination is also more successful with a 42% GIFT pregnancy rate and a 38% IVF pregnancy rate and a small early pregnancy loss of 14% (Abdalla *et al.* 1988). Rutherford *et al.* (1988) have recently reported a similar success rate with IVF using the long protocol. These factors minimize the overall cost for assisted conception management with more efficient use of resources and a more successful outcome.

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Programmed gamete intrafallopian transfer (GIFT)

R. G. BATES, P. M. FIELDING, K. S. LINDSAY, N. J. WHITE, D. K. EDMONDS

Summary. Thirty-three patients with unexplained infertility underwent a total of 42 programmed superovulation cycles in a gamete intrafallopian transfer (GIFT) programme. The date of oocyte retrieval was decided in advance and the cycle preceding oocyte collection was modified with norethisterone from mid-cycle until 14 days before the scheduled laparoscopy. This was followed by a fixed schedule superovulation regimen. Serum oestradiol, progesterone and luteinizing hormone were monitored and the data analysed retrospectively. A single ultrasound scan was performed on the day of laparoscopy to exclude ovulation. Thirty-eight GIFT procedures were performed, resulting in 11 (29%) clinical pregnancies of which four twin and four singleton pregnancies are continuing. There was a significant correlation between the oestradiol response pattern and the maturity of oocytes retrieved, the fertilization rate of supernumerary oocytes and the pregnancy rate. Programmed cycles may be conveniently combined with GIFT, and basic endocrinological monitoring can be used to identify cycles with a poor prognosis before laparoscopy.

In-vitro fertilization and embryo transfer (IVF-ET) were originally used for the treatment of couples with irreparable tubal damage. However, indications for IVF-ET have widened in recent times to include such disorders as endometriosis, cervical hostility, anti-sperm antibodies, oligozoospermia and unexplained infertility. Recently a simpler and less expensive procedure has been applied as an alternative to IVF-ET in patients with normal fallopian tubes, namely gamete intrafallopian transfer (GIFT) (Asch *et al.* 1986; Nemiro & McGaughey 1986).

The GIFT procedure involves laparoscopic aspiration of oocytes from ovarian follicles in hyperstimulated cycles, the preparation of a highly motile fraction of sperm, and deposition of recovered oocytes together with sperm in the ampulla of the fallopian tube. Pregnancy rates of between 15% (Nemiro & McGaughey 1986) and 40% (Matson *et al.* 1987) have been reported in relatively small series. Molloy *et al.* (1987) reported a clinical pregnancy rate of 27% in patients with mild endometriosis or unexplained infertility; this is twice the pregnancy rate obtained for such patients treated with IVF-ET in the same unit, suggesting the superiority of GIFT over IVF-ET for selected patients.

Whilst the GIFT procedure is considerably simpler than IVF-ET, the problem for many units wishing to provide this service has been the need for 'tailor-made' stimulated cycles which are complex and expensive. Facilities for intensive endocrinological and ultrasonographic monitoring are required on a daily basis, as are facilities for performing laparoscopies.

Recently Frydman *et al.* (1986) described a

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new appproach to follicular stimulation for IVF-ET using programmed oocyte retrieval. The date of oocyte retrieval was decided several months in advance and the cycle preceding oocyte recovery modified with a progestogen or the contraceptive pill. This was followed by fixed schedule ovulation induction without intensive monitoring. The pregnancy rate, number of oocytes per retrieval and oocyte maturity did not differ between programmed cycles and matched controls undergoing conventional 'tailor-made' cycles.

We report here the results of our first series of programmed cycles combined with GIFT and present an analysis of the correlation between endocrine response and outcome in terms of pregnancy rate, maturity of oocytes retrieved and fertilization rate of supernumerary oocytes.

Patients and methods

The series included 33 patients with unexplained infertility who underwent a total of 42 programmed superovulation cycles. All the couples had been infertile for ≥5 years (mean 7·2 years). There were 32 couples with primary infertility and one with secondary infertility. Female partners satisfied the following criteria: age ≤37 years (mean 32·8 years); normal background follicle-stimulating hormone (FSH), LH, prolactin and ovulatory luteal phase progesterone levels (>30 nM); normal laparoscopy and dye-tubation. Male partners all had normal semen analysis according to the criteria of WHO (1980) with absent antisperm antibodies. Ten couples had previously been treated unsuccessfully with IVF-ET despite a proven ability to fertilize.

The day of follicle aspiration was decided in advance and this was always planned for day 13 of the treatment cycle. The cycle preceding the treatment cycle was modified by giving norethisterone 10 mg daily from day 15 onwards, ceasing treatment 14 days before the date of follicle aspiration. The duration of norethisterone treatment varied from 10 to 25 days. On day 2, superovulation was started with clomiphene citrate (Serophene, Serono Ltd, Welwyn) 50 mg daily for 5 days, and concurrent Pergonal (Serono Ltd, Welwyn) 150 i.u. intramuscularly (i.m.) for 8 days. On day 11 of the cycle, 5000 i.u. human chorionic gonadotrophin (hCG) were given i.m., and laparoscopic oocyte retrieval and GIFT were performed 35 h later.

Blood was taken for serum oestradiol and LH estimations from day 8 to day 11 inclusive, although the results were analysed retrospectively and were not available at the time of GIFT. A single ultrasound scan was performed on the day of oocyte retrieval only to exclude recent ovulation. No measurements were taken.

A semen sample was obtained within 6 h of scheduled laparoscopy and allowed to liquefy for 30 min. Thereafter a motile sperm suspension was prepared by a modified migration–centrifugation method (Yates & De Kretser 1987). Briefly, motile sperm migrated from semen directly into a modified Earle's culture medium (Gibco, London) containing 10% heat-inactivated human serum and were then centrifuged (500 *g* for 5 min) and resuspended in fresh medium. The final concentration of actively motile sperm was adjusted to 4×10^6/ml by dilution with culture medium as necessary.

Oocytes were retrieved laparoscopically using a double-channel follicle aspiration needle (Wolf UK Ltd, Mitcham, Surrey). Where necessary, follicles were flushed with HEPES buffered Earle's culture medium supplemented with 5% human serum. Recovered oocytes were graded according to maturity as judged by expansion of the corona-cumulus complex and presence or absence of a first polar body (Asch *et al.* 1985). Oocytes were then kept in culture medium at 37°C until completion of the egg collection, when the four most mature oocytes were selected for tubal transfer.

The catheter used for tubal transfer was a Craft GIFT catheter (Rocket, London) with an end opening. Two oocytes in 0·025 ml of culture medium, and 100 000 actively motile sperm in a further 0·025 ml of culture medium were drawn up into the catheter with a sterile tuberculin syringe. The loaded catheter was passed through a purpose-made cannula into the abdominal cavity and then through the fimbrial ostium of the fallopian tube. The catheter was passed 1·5–2 cm down the fallopian tube and the contents were discharged into the ampullary region. When four oocytes were available a maximum of two oocytes was transferred to each tube. When there were three or less, all the oocytes were transferred to one side.

Where more than four oocytes were collected, the remaining oocytes were graded and pre-incubated in culture medium for 6–16 h, depending upon maturity, before insemination with 100 000 motile sperm per oocyte. Thereafter, oocytes were routinely examined for pronuclear formation and cleavage. Embryos developing as far as the blastocyst stage were cryopreserved.

Patients were seen again on day 18 (7 days after hCG) for serum progesterone estimation and 14 days after tubal transfer for a urine β-hCG pregnancy test. Patients with a positive urine pregnancy test attended again after a further 14 and 28 days for ultrasound scan. Clinical pregnancies were confirmed by the presence of a gestation sac, and a living fetus was confirmed by the presence of a fetal heart.

Oestradiol was measured by radioimmunoassay performed on ether-extracted plasma, using a polyclonal antiserum raised against oestradiol-6-carboxymethyl oxime linked to bovine serum albumin (Imperial Cancer Research Fund). A tritium tracer (Amersham International) was used and the bound fraction separated from the free by dextran-coated charcoal. The coefficients of variation (CV) (%) for this assay at points low, middle and high on the standard curve were: for between batch 7·7, 7·2 and 9·2, and for within batch 6·3, 6·1 and 7·8, respectively.

Luteinizing hormone (LH) was assayed using an adaptation of the Chelsea Radioimmunoassay kit (Ferguson *et al.* 1982) modified to give results within 3 h. The interassay CV values were 15·4 (low), 9·3 (middle) and 6·4 (high) and the intra-assay CV were 11·9, 7·1 and 5·2 respectively. An LH surge was defined as a level of more than 20 i.u./l after a normal background level of less than 10 i.u./l

Progesterone levels were measured with a NETRIA direct radioimmunoassay kit (North-East Thames Radioimmunoassay, London). The interassay CV values were 12·1 (low), 8·9 (middle) and 9·1 (high) and intra-assay 11·9, 7·1 and 5·2 respectively. Levels >30 nM were accepted as indicating good luteal phase progesterone production. During this study all specimens from individual patients were analysed within the same assay batch.

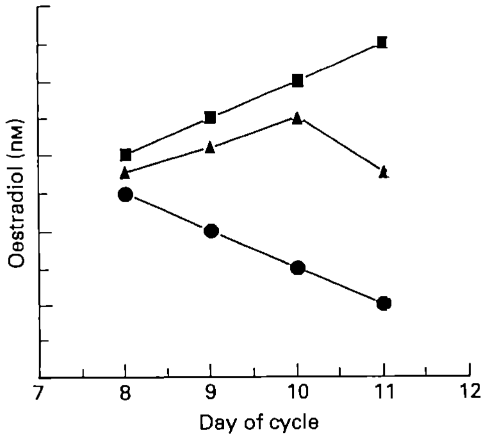

Fig. 1. Diagrammatic representation of oestradiol response patterns. ■, Type 1; ▲, type 2; ●, type 3.

Oestradiol response patterns were divided into three types using a modified version of the classification suggested by Jones *et al.* (1983) (Fig. 1). In type 1, oestradiol levels rise daily during Pergonal stimulation and continue to rise for 2 days before hCG administration; in type 2, oestradiol levels rise daily during Pergonal stimulation but fall on one or both days after discontinuation of stimulation before hCG administration; and in type 3, oestradiol levels fall during Pergonal stimulation.

The results were analysed statistically by the χ^2-test.

Results

Overall, 33 patients completed 42 programmed superovulation cycles. In four of these there was obvious evidence of ovulation on ultrasound scan on the scheduled day of laparoscopy, and hence 38 GIFT procedures were performed. At least one oocyte was collected and used for transfer in each patient. The average number of oocytes collected was 5·3 per laparoscopy and 3 or 4 oocytes were transferred in 37 of the 38 procedures.

Of the 38 laparoscopies and GIFT procedures, 11 (29%) resulted in clinical pregnancies. Eight pregnancies (21%) are continuing, these comprise four twin and four singleton pregnancies. Of the three pregnancies lost, one was an anembryonic gestation at 8 weeks and the other two ended in miscarriage at 7–8 weeks.

Table 1 summarizes the endocrine response patterns and outcome of treatment. Eleven pregnancies occurred in the type 1 response group compared with none in either the type 2 or 3 group ($P<0·05$). It is also evident that the occurrence of an LH surge was not confined to one oestradiol response pattern, and only four of the seven patients who had a premature LH surge (18% of treatment cycles) had corresponding ultrasound evidence of ovulation resulting in cancellation of oocyte retrieval.

Table 2 shows that whilst the number of oocytes retrieved per laparoscopy does not differ significantly between groups, there were significantly more mature oocytes (grade 4/5) recovered with type 1 response compared with type 2 or 3 ($P<0{\cdot}01$). All 27 GIFT procedures involving a type 1 response pattern had at least one grade 4 or 5 oocyte transferred compared with five out of nine associated with a type 2 oestradiol response.

Table 3 summarizes the outcome of attempts to fertilize supernumerary oocytes by in-vitro fertilization. Oocytes retrieved from cycles with a type 1 response performed significantly better in terms of fertilization rate ($P<0{\cdot}01$) than those retrieved from type 2 cycles. There were no supernumerary oocytes in type 3 cycles.

The mean plasma progesterone level 7 days after the administration of hCG was 143 (SD 64) nM. There was no significant difference beween the mid-luteal progesterone levels in the three response groups.

There was no relation between the length of progestin therapy and the occurrence of any particular type of endocrine response or pregnancy.

Discussion

The suitability of the GIFT technique for treatment of idiopathic infertility has already been

Table 1. Patterns of oestradiol response and outcome of treatment

Response pattern	No of cycles	No of laparoscopies	No. of pregnancies
Type 1			
LH−	25	25	11
LH+	5	2	0
Type 2			
LH−	8	8	0
LH+	2	1	0
Type 3			
LH−	2	2	0
LH+	0	0	0
Total	42	38	11

LH+ and LH− indicate the presence or absence of a premature luteinizing hormone peak.
There were signficantly more pregnancies associated with the type 1 response pattern ($P<0{\cdot}05$)

Table 2. Oestradiol response patterns related to oocyte maturity

Response pattern	No. of laparoscopies	Frequency of oocyte grade*						No. of oocytes retrieved	
		L	1	2	3	4	5	Total	Per laparoscopy
Type 1	27	5	0	5	56	78	2	146	5·4
Type 2	9	6	0	8	23	15	0	52	5·8
Type 3	2	0	0	1	2	1	0	4	2·0
Total	38	11	0	14	81	94	2	202	2·0
		(5%)	—	(7%)	(40%)	(47%)	(1%)		

* Oocyte maturity grade: L, luteinized; 1, germinal vesicle present; 2, compact corona/cumulus complex (c/c); 3, partially expanded c/c; 4, expanded c/c; 5, first polar body present.
There were significantly more mature pre-ovulatory oocytes (grades 4 or 5) associated wih the type I response pattern ($P<0{\cdot}01$).

Table 3. Fertilization and development of supernumerary oocytes

Response pattern	Total no.	Fertilized	Cleaved	Blastocyst
Type 1	43	30 (70%)	28 (93%)	5 (17%)
Type 2	17	5 (29%)	4 (80%)	0

The fertilization rate of supernumerary oocytes retrieved during type 1 cycles was significantly higher ($P<0{\cdot}01$).

established (Asch *et al.* 1986; Nemiro & McGaughey 1986; Matson *et al.* 1987; Molloy *et al.* 1987). The usefulness of this technique in male factor infertility remains to be established and the results of a large multicentre trial are awaited. However, until now all the series of GIFT published have employed 'tailor-made' stimulated ovarian cycles rather than a fixed stimulation regimen.

The concept of fixed-schedule ovulation induction is not new (Templeton *et al.* 1984; Braude *et al.* 1984), though it was originally employed to provide human oocytes for research purposes. More recently this technique has been employed in a clinical IVF programme (Frydman *et al.* 1986) and although the number of patients was small, the number of oocytes generated per laparoscopy, the fertilization rate and the pregnancy rate did not differ from patients undergoing 'tailor-made' cycles.

A more complex form of programmed stimulation for IVF-ET has been described recently (Zorn *et al.* 1987) using norethisterone in the pretreatment cycle and a combination of an LHRH analogue and Metrodin in the stimulated cycle. Whilst this would obviate the problem of premature LH surges and largely eliminate operating at weekends, the treatment is expensive and the precise date of laparoscopy cannot be decided in advance.

Before introducing programmed GIFT into our department we had been unable to provide a GIFT service because theatre time could not be made available on a daily basis. The reported success of programmed cycles with IVF-ET and the availability of an operating theatre one afternoon each week for GIFT procedures led us to introduce programmed GIFT. To our knowledge this is the first time a fixed stimulation protocol has been employed in a GIFT programme.

Whilst cycle monitoring was not used by Frydman *et al.* (1986) and the results of programmed IVF compared favourably with intensively monitored cycles for IVF, other studies of oestradiol response in the follicular phase of IVF-ET cycles have identified trends suggesting the superiority of one pattern of response but no statistically significant difference was identified (Jones *et al.* 1983; Diamond *et al.* 1985; Pellicer *et al.* 1987). To establish whether cycle monitoring could be usefully employed in programmed cycles for GIFT, endocrinological monitoring was performed and the results analysed retrospectively.

As our intention was to keep cycle monitoring as simple as possible and many of our patients live a considerable distance from the hospital, the first three blood samples (days 8, 9 and 10) were taken by the patient's general practitioner where possible. The blood samples were stored at home in a refrigerator and brought to the hospital on day 11 when a final blood sample was taken. All four blood samples were then assayed in the same batch to avoid interassay variation. Oestradiol response patterns were classified using a simplified version of the classification described by the Norfolk group (Jones *et al.* 1983).

Retrospective analysis of endocrine data after 42 superovulation cycles and 38 GIFT procedures shows a significant correlation between the pattern of oestradiol response and the maturity of oocytes retrieved, the fertilization rate of supernumerary oocytes and the occurrence of pregnancy. It seems likely that the superiority of a type 1 response in terms of pregnancy rate is at least in part a result of the high proportion of mature oocytes retrieved in such cycles. Since oocytes are effectively incubated with sperm immediately using the GIFT technique, oocyte maturity is probably important, as it is known that immature oocytes have poor fertilization rates *in vitro* which can only be improved by prolonged pre-incubation in culture medium free of sperm (Veeck 1986). Furthermore, Nemiro & McGaughey (1987) have found pregnancy rates in their GIFT programme to be highly correlated with the number of mature oocytes obtained at laparoscopy and with the number of oocytes transferred.

It has been suggested that GIFT should not be performed on all couples with normal fallopian tubes unless the ability to fertilize has been demonstrated. This information may be unobtainable using the GIFT technique as some oocytes are replaced and then the only proof of fertilization is pregnancy (Quigley *et al.* 1987).

Matson *et al.* (1987) reported a pregnancy rate of 40% in normozoospermic couples and 16·7% in oligozoospermic couples treated with GIFT, but he found no correlation between the success or failure of supernumerary oocytes to fertilize *in vitro* and subsequent pregnancy. This was attributed to the low numbers of supernumerary oocytes and oocyte selection criteria for GIFT.

In this series of normospermic couples, pregnancy and fertilization of supernumerary oocytes was related to the quality of the follicular phase as judged by the pattern of oestradiol response and maturity of the oocytes obtained.

Whilst it would seem logical to treat couples with suspected male factor infertility with IVF-ET as a diagnostic and therapeutic procedure, there is no evidence to support the idea that normospermic couples should be treated similarly. Indeed, GIFT appears to have a higher success rate than IVF in the treatment of idiopathic infertility (Molloy *et al.* 1987). It is advantageous, however, to carry out GIFT in an IVF unit as any spare oocytes generated can be fertilized *in vitro* and cryopreserved.

In conclusion, GIFT appears to be a relatively simple and successful form of treatment for idiopathic infertility. Programmed superovulation cycles are appropriate to the GIFT technique and have considerable advantages in terms of convenience. We would suggest that cycle monitoring in the form of daily oestradiol estimations from day 8 to day 11 inclusive should be performed and an ultrasound scan carried out on the day of laparoscopy. Only those patients with a type 1 oestradiol response and no ultrasound evidence of ovulation should proceed to laparoscopy.

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Transvaginal pulsed Doppler ultrasound assessment of blood flow to the corpus luteum in IVF patients following embryo transfer

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Summary. The waveforms of vessels supplying the ovaries of women on an in-vitro fertilization (IVF) programme were studied using transvaginal B-mode and Doppler ultrasound. There were 125 scans recorded in 65 women at weekly intervals from 3 days after embryo transfer or 5 days after gamete intrafallopian transfer (GIFT) until confirmation of pregnancy or onset of menses. At each examination the signals obtained from vessels supplying the ovaries were recorded and quantified using a resistance index (RI). Fifteen patients became pregnant of whom one has subsequently miscarried. There was a highly significant difference in the RI values between patients who became pregnant and those who did not; no patient who became pregnant had a RI greater than 0·5. Oestrogen to progesterone ratios were calculated in the subgroup of non-pregnant patients and there was no correlation between these values and the RI values. This new technique enables prediction of IVF treatment failure earlier than has been reported previously and may reflect the inadequacy of the corpus luteum.

The development of high resolution real-time B-mode ultrasound has provided a method for the non-invasive study of reproductive structural changes of ovarian follicles. In contrast, Doppler ultrasound offers a non-invasive means of studying blood flow patterns and hence allows identification of functional changes. The use of Doppler ultrasound in the clinical assessment of vascular disease, notably in the head and neck, has already been established (Atkinson & Woodcock 1982; Woodcock *et al.* 1983) and this work has suggested that each artery appears to have its own characteristic Doppler waveform which may be modified by disease. Taylor *et al.* (1985) and Taylor & Burns (1985) have demonstrated characteristic waveforms for ovarian, uterine and iliac vessels and also the changes in these waveforms associated with hormonal

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changes. Transvaginal ultrasound produces images of better resolution than transabdominal ultrasound, primarily through better preservation of beam pattern and utilization of higher frequencies. It was expected that the transvaginal approach to Doppler would offer the same benefits as for imaging and this approach was utilized. The purpose of this study was to assess the waveforms of vessels supplying the ovaries in patients on an in-vitro fertilization (IVF) programme following their embryo transfer (ET) or gamete intrafallopian transfer (GIFT) in order to determine whether a difference in waveform patterns existed between those who would and those who would not become pregnant.

Patients and methods

Before the study was started the maximum acoustic output of the equipment was measured in imaging and pulsed Doppler mode, and at each intensity specified by the United States Food and Drug Administration (1987) was measured to be far less than the allowed limits (Table 1).

Overall, 125 scans were performed on 65 volunteer patients who were recruited from our IVF programmes after giving informed consent. All women had received ovarian stimulation with clomiphene citrate and human menopausal gonadotrophins (hMG) and their ovulation had been induced with 5000 i.u. human chorionic gonadotrophin (hCG) according to our usual protocol (O'Neill *et al.* 1985*a*). Each patient underwent transvaginal B-mode imaging and Doppler ultrasonic scanning of the pelvis, the first examination being performed 3 days after embryo transfer or 5 days after GIFT. Subsequent examinations were performed weekly until pregnancy was confirmed by an elevated serum hCG or until menses ensued. Examinations used a Micro Imager 1000 mechanical sector transducer (Ausonics Pty Ltd, Lane Cove, Australia) fitted with a 7·5 MHz transvaginal probe linked to a 6 MHz pulsed Doppler system (Vingmed Pty Ltd, Horten, Norway). A 3–5 mm sample volume was used. Both Doppler waveforms and real-time images were recorded for subsequent analysis. Initially, scans were recorded on videotape from which polaroid images could be obtained. Later in the study, images were recorded on X-ray film using a multi-format camera. At each examination the ovaries were identified using real-time ultrasound and their size noted as well as the number, appearance and size of any cysts. Doppler scanning was then performed by moving the sample volume systematically over the supero-lateral margin of each ovary and through its substance until signals from the ovarian artery and/or the vascular supply to the corpus luteum had been obtained. These signals were identified by their waveforms as described previously (Taylor *et al.* 1985; Taylor & Burns 1985). Once identified, the signal quality was maximized by slight adjustments to the sample volume.

Table 1. Ultrasound intensity in water

	B-mode 7·5 MHz	Pulsed Doppler 6 MHz	FDA limits
SPTA mW/cm²	19	30	180
SPPA W/cm²	161	2	350
Im W/cm²	199	2	550

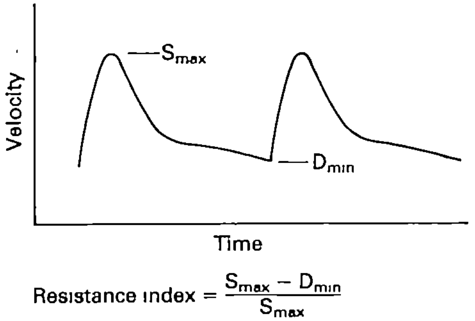
Signal analysis

Photographic record of each waveform was examined by a single investigator (M.B.McS.) immediately after each investigation and without any knowledge of the patient's pregnancy status. Because it was not always possible to see the vessel in which the flow was being recorded, and hence to measure the angle between the ultrasound beam and the vessel, estimation of absolute velocity and volume of flow was not possible. However, angle-independent indices have been described which assess waveforms as a reflection of distal impedance (Woodcock *et al.* 1974). In this study the resistance index (RI) (Planiol & Pourcelot 1974) was used to quantify the waveforms (Fig. 1).

The lowest RI recorded from either ovary was the value used in the data analysis.

Endocrinology

In each patient the oestradiol to progesterone ratio was calculated on the first day after embryo transfer or third day after GIFT (O'Neill *et al.* 1985*b*). This ratio was later compared with the RI in the non-pregnant group of patients.



$$\text{Resistance index} = \frac{S_{max} - D_{min}}{S_{max}}$$

Fig. 1. Resistance index (Planiol & Pourcelot 1974).

Results

A total of 125 scans was recorded in 65 patients of whom 15 became pregnant. One has subsequently had a miscarriage. Table 2 shows the mean RI in the pregnant and non-pregnant groups at both day 3 post-ET (day 5 post-GIFT) and day 10 post-ET (day 12 post-GIFT). The difference between the resistance indices of those patients who became pregnant and those who did not is highly significant ($P<0{\cdot}0001$) and no patient who became pregnant had a RI greater than 0·5, although not all patients with a RI below 0·5 became pregnant. In the one patient who miscarried the RI gradually rose over several scans and a value above 0·5 was found before the miscarriage.

There was no correlation between patients with high luteal phase peripheral oestradiol to progesterone ratios and RI in the non-pregnant patients.

Discussion

The low success rate of IVF programmes remains a source of concern (Bartels 1987). The clinical pregnancy rate in IVF programmes usually approaches 19 per 100 treatment cycles (Baber *et al.* 1988; Feichtinger & Kemeter 1986) with miscarriage rates of 25–35% (Jones *et al.* 1983). Clearly the causes of a low success rate may be maternal, such as increasing maternal age, a poor obstetric history, high levels of follicular oestrogens and luteal inadequacy, or embryonic, such as the number and quality of embryos transferred (Edwards & Steptoe 1983). Attempts have been made to assess luteal phase inadequacy as a cause of failed implantation using endocrine measures, but with little success (Trotnow *et al.* 1982; Feichtinger *et al.* 1982). In the present study it was not always possible using real-time scanning to differentiate the corpora lutea from residual cysts, although in general the corpora lutea contained more echogenic material. The Doppler waveforms obtained from the ovaries were similar to those described by Taylor *et al.* who suggested that a high diastolic blood flow was present in the ovary containing the corpus luteum and a low diastolic blood flow existed in the inactive ovary (Taylor *et al.* 1985; Taylor & Burns 1985) (Figs 2 & 3).

The use of transvaginal Doppler allows accurate placement of the sample volume. Using Doppler waveforms we have been able to distinguish the ovary containing the active corpus luteum from the inactive ovary and by measuring the RI of these waveforms we have devised a numerical index which appears to predict pregnancy failure as early as 3 days after embryo transfer. Since an increasing RI reflects some reduction in the blood flow distal to the point at which the Doppler recording was made, namely in the corpus luteum, one might postulate that this reflects a reduction in corpus luteum function which is incompatible with pregnancy.

Not all patients with a RI consistently below 0·5 became pregnant. This presumably indicates that these patients failed to become pregnant for reasons other than the adequacy of their ovarian blood flow.

A RI value of 1·0 appears to be a more accurate predictor of pregnancy failure than the oestradiol to progesterone ratio. However, we have been unable to demonstrate a correlation between the oestradiol to progesterone ratios and treatment outcome in this non-pregnant group, which suggests that the problem may lie with the embryos.

Table 2. Resistance index (RI) after embryo transfer (or GIFT) in women who became pregnant and women who did not

	RI of corpus luteum after embryo transfer	
Patient group	At 3 days	At 10 days
Pregnant	0·46 (0·009)*	0·45 (0·017)*
Non-pregnant	0·83 (0·026)	0·90 (0·026)

Values shown are mean (SE).
Significance of difference between pregnant and non-pregnant groups * $P<0{\cdot}0001$, Student's *t*-test.

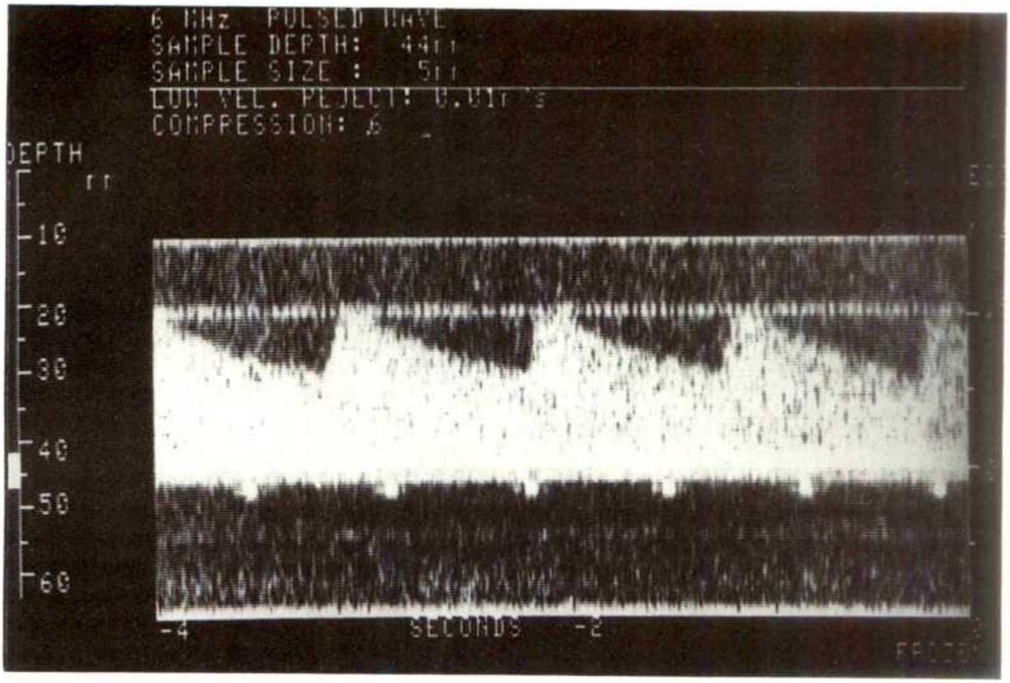


Fig. 2. Low impedance Doppler waveform characteristic of an active corpus luteum.

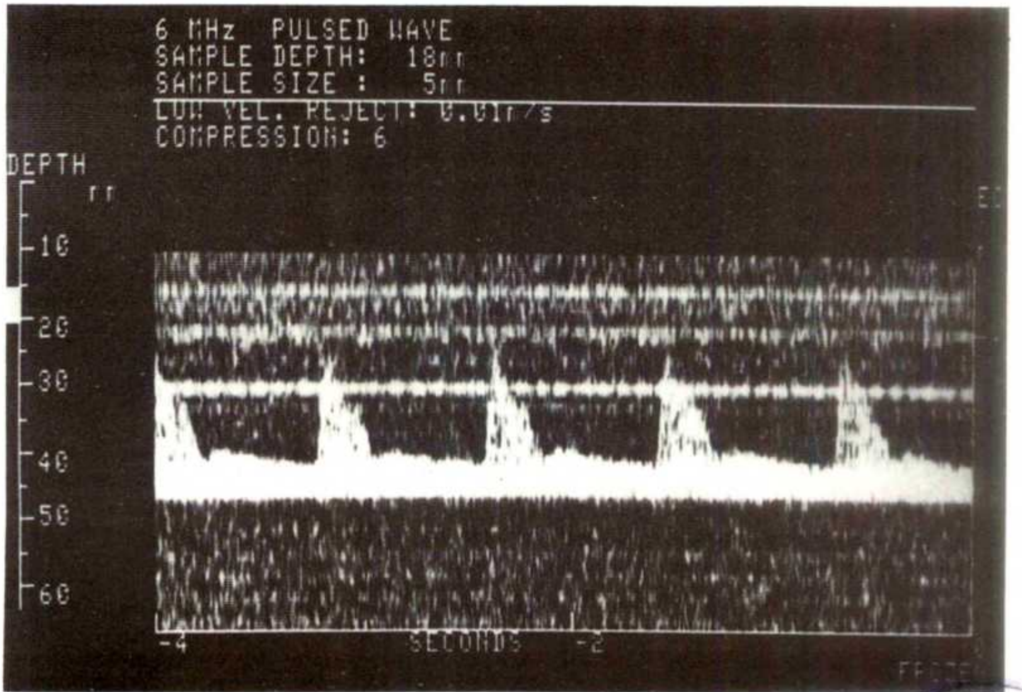


Fig. 3. High impedance Doppler waveform characteristic of an inactive ovary.

Assessment of embryo quality and function is the next stage of this continuing study.

In conclusion we believe that transvaginal pulsed Doppler ultrasound enables prediction of IVF treatment failure at an earlier stage than has previously been reported and this could reflect inadequacy of the corpus luteum.

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The prognostic and therapeutic value of second-look laparotomy in advanced ovarian cancer

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Summary. Of 267 patients with ovarian cancer FIGO stages III and IV, 157 underwent second-look laparotomy after combination chemotherapy consisting of cis-platinum and cyclophosphamide with and without doxorubicin. At second-look operation 45% had macroscopic tumour, 15% microscopic tumour, and 40% complete pathological response. Survival 3 years after second look was: complete pathological response 74%; microscopic disease 24%; visible tumour <1 cm 28%; visible tumour >1 cm 17%; negative cytology 59%; and positive cytology 18%. Of the patients with macroscopic tumour, 29% had all visible tumour removed at second look. Three-year survival for these patients was about 45%.

The treatment of choice for patients with epithelial advanced ovarian cancer is combination chemotherapy. Complete clinical response is achieved in about 50%, but complete pathological response only in about 25% of the patients (Ozols & Young 1984; Piver 1984). The only method to assess pathological response is a second-look laparotomy, which has become a standard procedure in the treatment of advanced ovarian cancer responding to chemotherapy (Roberts *et al.* 1982).

Surgical re-exploration has the following potential benefits:

(1) Avoiding premature cessation of chemotherapy when residual, but not clinically detectable, disease is still present (Podratz *et al.* 1985).

(2) Allowing termination of chemotherapy as soon as possible and thereby reducing the acute toxicity and potential long-term risk of late toxicity (Podratz *et al.* 1985).

(3) Making possible secondary resection in patients with residual tumour (Wils *et al.* 1986).

(4) Early and more reliable evaluation in clinical trials.

Patients and methods

A total of 267 patients with epithelial ovarian cancer FIGO stages III and IV was allocated to a Danish multicentre study in the 3-year period from 1 September 1981. After informed consent the patients were randomized to combination chemotherapy consisting of cyclophosphamide and cis-platinum with (CAP) and without dox-

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orubicin (CP). The doses per cycle were: cyclophosphamide 500 mg/m^2, cis-platinum 60 mg/m^2 and doxorubicin 40 mg/m^2. Chemotherapy was given every 4 weeks, if tolerated for 12 cycles. Before randomization all patients underwent surgical staging, radical surgery if possible or debulking operation. Detailed description of the patients has been given previously by Bertelsen *et al.* (1987). One month after the last cycle of chemotherapy a second-look laparotomy was performed on patients in clinical response. All operations were performed or directed by senior surgeons. With all the patients under general anaesthesia, the operations were performed through a midline incision. After the abdomen had been entered, washings from the pelvic cavity and cytological scrapings from left and right subdiaphragmatic area were taken. The whole abdominal cavity was carefully inspected and biopsies taken from any visible disease or suspicious area. If not primarily performed, oophorectomy, hysterectomy and omentectomy were carried out, if possible. In patients without macroscopic disease, multiple biopsies were taken, in the majority of the patients from four sites in the pelvis, two sites lateral to the colon and from residual omentum, with a mean number of five biopsies per patient. In patients with macroscopic tumour an attempt to remove all tumour was made. Therapy after a positive second look was individualized and consisted mostly of single agent chemotherapy given orally. A few patients had intravenous combination chemotherapy or intraperitoneal single agent chemotherapy.

Assessment of response and survival

If all histological and cytological specimens were negative, the patient was considered to be in complete pathological remission. If microscopic examination revealed tumour tissue or tumour cells, in spite of normal macroscopic appearance, the status was termed 'microscopic disease'.

Table 1. Distribution of second-look operation and treatment regimens in 267 patients with ovarian cancer

	Treatment regimen	
	CP (n = 135)	CAP (n = 132)
Second-look operation	73	84
No second-look operation	62	48
Because of:		
Death	33	34
Progression	16	7
Operation contraindicated	5	3
Operation refused	8	4

CP, Cyclophosphamide + cisplatin; CAP, cyclophosphamide + cisplatin + doxorubicin.

Survival. This was estimated by Kaplan–Meier plots (Kaplan & Meier 1958) and analysed for differences by the use of the Mantel–Haenszel test (Mantel & Haenszel 1959). Survival was calculated from the date of second-look laparotomy to the date of last observation or to the date of death.

Results

Follow-up information on the 267 randomized patients has been obtained until 1 January 1987.

Of the 267 patients, 157 (59%) had a second-look laparotomy, with time of observation after the second-look laparotomy from 14 to 52 months. In 110 patients no second-look operation was performed because of death, progression of disease or other reasons (Table 1).

The mean interval between initial staging operation and second-look laparotomy was 357 days (range 189–616 days). Most of the 157 patients who had a second-look operation had full chemotherapy, with 92% having 10 cycles or more.

Of the 157 patients, 70 (45%) had macroscopic tumour, and 87 (55%) had no macroscopic tumour at second-look laparotomy. Twenty-four of these revealed tumour at microscopy and one had a malignant pleural exudate. This patient has been excluded from further assessment (Table 2).

Secondary debulking was performed to tumour size less than 1 cm in 32 of the 70 patients with macroscopic tumour. Twenty ended up with no macroscopic tumour, of whom 11 had negative cytology and nine positive cytology (Table 2).

Sixty-two (40%) of the 86 without macroscopic tumour were in complete pathological response. Among the 24 patients with microscopic disease, 12 had both positive biopsies and cytology, five had positive biopsies and negative cytology and one had positive biopsies and no cytological examination. The remaining six patients had only positive cytology (Table 2).

Overall, 135 patients had cytological examination of both diaphragmatic scrapings, and either ascites or peritoneal washings. Twenty-two had cytological examination only from

Table 2. Tumour status before and after second-look operation

		At the end of second look			
		Macroscopic tumour		No macroscopic tumour	
		>1 cm	≤1 cm	Positive cytology	Negative cytology
At the beginning of second look					
Macroscopic tumour	70				
>1 cm	50	25	12	6	7
≤1 cm	20	—	13	3	4
No macroscopic tumour	86				
Microscopic tumour	24	—	—	18	6*
Complete pathological response	62	—	—	—	62

* Five patients with positive biopsies and negative cytology, and one patient with positive biopsy, no cytological examination.

either scrapings or ascites/washings. Positive cytology of diaphragmatic scrapings was always associated with positive ascites/peritoneal washings. Positive cytology in ascites/washings, however, was not always associated with positive scrapings. Out of 50 patients with positive cytology in ascites or peritoneal washings 17 patients (34%) had no tumour cells in scrapings from the diaphragm.

The relation between complete pathological response and the findings at primary operation is shown in Tables 3–5.

At primary staging, 134 (86%) were in stage III and 22 (14%) in stage IV. At second look 57 (43%) of the patients in FIGO stage III had complete response compared with five patients (23%) in FIGO stage IV. The difference was not statistically significant. In relation to the histological types complete pathological response varied between 67% in mucinous carcinoma and 26% in undifferentiated carcinoma, with 40% in the most common types of serous and of endometrioid carcinoma. The differences were not statistically significant (Table 3). No relation between histological grade and complete pathological response was seen (Table 4).

Residual tumour after primary operation was correlated with the findings at second look (Table 5). Of the 18 patients with no residual tumour 12 had complete pathological response (67%) and of 34 patients with residual tumour smaller than 1 cm 19 had complete pathological response (56%). On the other hand, of the 98 patients with residual tumour bigger than 1 cm, only 28 (29%) had complete pathological response. The difference between no residual tumour or residual tumour ≤1 cm and residual tumour >1 cm was statistically different ($\chi^2 = 12{\cdot}45$, $P<0{\cdot}001$).

Survival after second look related to surgical/pathological characteristics at re-exploration

Figure 1 shows survival after second look in relation to the findings at the beginning of the re-exploration. Survival of patients in complete pathological response was superior to that of patients with any kind of residual tumour. Patients in complete pathological response have a 3-year survival rate of 74% compared with patients with microtumour (24%), patients with residual tumour less than 1 cm in diameter (28%) and patients with residual tumour larger than 1 cm (17%). The difference between survival of patients in complete pathological response and that of patients with any kind of residual tumour was significant ($\chi^2 = 47{\cdot}03$, $P<0{\cdot}001$).

Seventy patients had macroscopic tumour at the beginning of second look. In 50 patients the tumour was >1 cm in diameter and in 20 patients the tumour was ≤1 cm. In 32 patients it was possible to reduce the residual tumour (Table 2). Patients with macroscopic residual tumour at the beginning of second look were analysed in four groups according to the status after secondary debulking. (1) No residual macroscopic tumour, negative cytological investigations, survival at 3 years after second look, 44%. (2) No residual macroscopic tumour but positive cytology, survival at 3 years after second look, 47%. (3) Macroscopic residual tumour ≤1 cm in diameter,

Table 3. Complete pathological response in relation to histological cell type

		Complete pathological response	
Histology	No. of patients	No.	%
Serous carcinoma	94	38	40
Mucinous carcinoma	6	4	67
Endometrioid carcinoma	30	12	40
Clear cell carcinoma	2	1	50
Undifferentiated carcinoma	19	5	26
Other	5	2	40
Total	156	62	

Table 4. Complete pathological response in relation to histological grade

		Complete pathological response	
Histological grade	No. of patients	No.	%
I	31	16	52
II	37	12	32
III	65	22	34
Unknown	23	12	52
Total	156	62	

Table 5. Complete pathological response in relation to residual tumour at primary surgery

		Complete pathological response	
Original residual tumour	No of patients	No	%
No residual tumour	18	12	67
Residual tumour ≤1 cm	34	19	56
Residual tumour >1 cm	98	28	29
Unknown	6	3	50
Total	156	62	

$\chi^2 = 12{\cdot}45$.

survival at 3 years after second look, 13%. (4) Residual tumour >1 cm, survival at 3 years after second look, 11% (Fig. 2).

There was a statistically significant difference between patients without macroscopic residual tumour after secondary debulking and patients with macroscopic tumour ($\chi^2 = 11{\cdot}17$, $P<0{\cdot}005$). Figure 3 shows survival after second look in relation to positive/negative cytology of ascites or peritoneal washings. Survival of patients with negative cytology was 59% at 3 years and survival of patients with positive cytology 18%; the difference was statistically significant ($\chi^2 = 54{\cdot}95$, $P<0{\cdot}001$).

Discussion

The present study shows that the size of residual tumour at original surgery significantly influ-

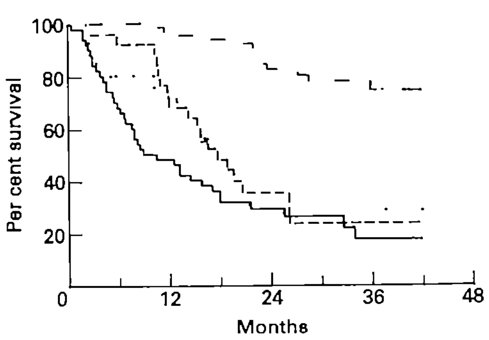

Fig. 1. Kaplan–Meier estimate of survival in 157 second-look operated patients, 62 in complete pathological response (CPR) (— · · ·), 24 with microtumour (- - -), 20 with macrotumour ≤1 cm (· · · ·) and 50 with macrotumour >1 cm (——).

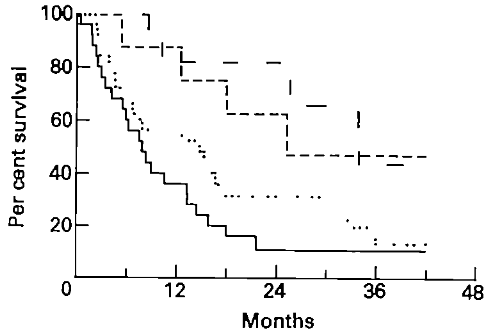

Fig. 2. Kaplan–Meier estimate of survival after secondary debulking, in 11 patients without macroscopic tumour and negative cytology (CPR) (— · · ·), in nine without macroscopic tumour but positive cytology (microtumour) (- - -), 25 with persistent visible tumour ≤1 cm (· · · · ·) and 25 with persistent visible tumour >1 cm (——).

ences the rate of complete pathological response. Patients with residual tumour ≤1 cm in diameter have a rate of complete pathological response of 60%; patients with residual tumour >1 cm a rate of 29%. There was a tendency towards a higher rate of complete pathological response for patients with FIGO stage III than for FIGO stage IV and also a tendency of better response for patients with mucinous carcinoma than for patients with serous, endometrioid or undifferentiated carcinoma. The differences are, however, not statistically significant.

These results are in agreement with recent studies (Mead *et al.* 1984; Cain *et al.* 1986; Podratz *et al.* 1985; Neijt *et al.* 1984), which also have shown a relation between the small-sized residual tumour at primary surgery and the rate of complete pathological response. Some of these papers also showed a relation between FIGO stage and the rate of complete pathological response (Podratz *et al.* 1985; Smirz *et al.* 1985; Neijt *et al.* 1984).

In the present study tumour cells in material scraped from the diaphragm were always associated with tumour cells in ascites or peritoneal washings, but one-third of the patients with positive ascites or peritoneal washings had negative diaphragm scrapings; the examination of diaphragm scrapings thus had low diagnostic value.

Patients in complete pathological response have a survival at 3 years of 74%, this corresponds well with the data of several other studies (Louie *et al.* 1986; Cohen *et al.* 1983; Raju *et al.* 1982; Podratz *et al.* 1985; Stuart *et al.* 1982). Survival of patients in complete pathological response is superior to that of patients with residual tumour, even microscopic tumour. Some studies (Neijt *et al.* 1984; Podratz *et al.* 1985) on the contrary found a similar survival for patients with complete pathological response and for patients with microscopic tumour at second look. This may be explained by a difference in the number of cycles of chemotherapy which numbered 12 in the present study compared with six in the studies reported by Neijt *et al.* (1984) and Podratz *et al.* (1985).

It was possible to perform secondary resection in several patients so that 20 ended up without macroscopic tumour. With or without positive cytology these patients had a relatively high survival rate, 47% and 44% 3 years after second look. This is in contrast to the survival rate of 24% in patients with microtumour at the beginning of second look. Our data correspond well with the findings of Sigurdsson (1982), Wils *et al.* (1986) and Podratz *et al.* (1985) and suggest that secondary debulking may have an impact on survival. But the data must be interpreted with caution because of the small number of patients, and it may be that the patients with residual tumour, removable at second look, represent a subgroup with a more favourable prognosis. To determine if secondary debulking has any impact on long-term survival a more detailed analysis of the patients with macrotumour at second look and of the treatment after second look is needed. This analysis is in progress.

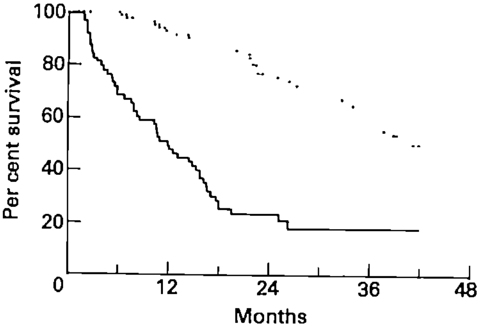

Fig. 3. Kaplan–Meier estimate of survival in patients with negative cytology (· · · · ·) and in patients with positive cytology (——) of ascites or peritoneal washings examined at second-look laparotomy.

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Sodium–renin–aldosterone relations in normal and hypertensive pregnancy

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Summary. To examine the short-term regulation of sodium excretion, plasma volume, and the renin-aldosterone system in pregnancy, women in their first pregnancy received either a high-salt (HS) (250 mmol/day) or a low-salt (LS) (20 mmol/day) diet for 7 days during the second and third trimester and after delivery (total 213 studies). Twenty women, studied while normotensive during mid-pregnancy, developed pregnancy-induced hypertension (PIH) in the third trimester. There was slightly greater difficulty adapting to sodium depletion (LS diet) during normal pregnancy compared with postpartum. The final mean values for sodium excretion were 27 (SE 2), 28 (SE 3) and 14 (SE 4) mmol/day in the second and third trimester and postpartum respectively. Sodium excretion with the HS diet was similar at all stages and plasma volumes were maintained as effectively during pregnancy as after delivery following both diets. Plasma renin activity (PRA) and aldosterone concentration rose and fell significantly following the LS and HS diets but the sensitivity of renin response to changes in salt intake was blunted during normal pregnancy. Women who later developed PIH, when studied whilst normotensive, failed to stimulate plasma aldosterone after salt depletion in their second trimester and did not exhibit the 'sodium-independent' component of PRA seen in continuously normotensive subjects at this stage.

The renin–aldosterone (R–A) system is a major determinant of sodium balance in pregnancy, opposing the natriuretic effects of filtered sodium, progesterone, arginine vasopressin (Brown & Gallery 1986), atrial natriuretic factor (Hirai *et al.* 1986), prostaglandins (Lewis *et al.* 1980), kallikrein (Karlberg *et al.* 1984) and other factors. The net result of this interplay between sodium regulatory factors is gradual sodium retention of approximately 900 mmol by the end of gestation (Chesley 1978) with attendant plasma volume expansion (Gallery *et al.* 1979).

Recently (Brown *et al.* 1987*a*) we have demonstrated that although the components of this system are stimulated markedly compared with the non-pregnant state, both renin and aldosterone respond physiologically about these new 'set-points' during pregnancy. Furthermore, these studies showed that the interrelations between sodium balance and this system alter as pregnancy progresses.

The present studies were designed to deter-

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mine: (i) whether the relations between sodium intake and plasma renin activity (PRA) differ from pregnancy to postpartum, and (ii) whether these relations are different from normal during mid-pregnancy in primigravidas who subsequently develop pregnancy-induced hypertension (PIH).

Patients and methods

The study included 142 white European women in their first pregnancy with no previous history of hypertension, cardiac or renal disease; they were allocated randomly to high-salt (HS) ($n = 70$) or low-salt (LS) ($n = 72$) dietary groups. The women were recruited into the study between 16 and 22 weeks gestation from a single 'booking-in' clinic where all pregnant women who are to be delivered at Royal North Shore Hospital, Sydney, are registered. Inclusion criteria were: first pregnancy, absence of pre-existing hypertension, renal or cardiac disease, normal blood pressure at first presentation, taking no medication which might influence sodium and/or water excretion. Random allocation to dietary groups was made after inclusion in the study by drawing from a box of equal numbers of cards labelled 'high-salt' or 'low-salt'. All women were studied whilst normotensive and it was predicted on the basis of previous studies of this population that approximately 17% would develop PIH in the third trimester (Gallery *et al.* 1979). PIH was defined as a resting diastolic (phase IV) blood pressure >90 mmHg after 24 h rest in hospital, developing after 20 weeks gestation in a primigravida with no known history of hypertension, and returning to normal within 3 months after delivery.

The study protocol for these subjects has been described in detail previously (Brown *et al.* 1987*a*). Briefly, the women in the HS group were allocated to ingest a diet containing approximately 250 mmol sodium per day for 1 week. Urine was collected for 48 h before starting the diet and for the final 48 h of the diet for (paired) measurements of urinary sodium, creatinine, and aldosterone excretion. At the commencement (day 0) and completion (day 7) of the diet blood was collected for measurement of plasma renin activity (PRA) and plasma volume, body-weight and blood pressure (BP) were measured. All subjects were studied once during the second trimester (22–26 weeks gestation) and some were studied again in the third trimester (32–36 weeks gestation) and again postpartum (after 3 months); at each study the women had the same diet as during their mid-trimester study.

Dietary compliance was assessed not by final urinary sodium excretion (as this would have *assumed* exact sodium balance to have taken place by 7 days) but by the direction of change of body-weight and by weighed food recordings, as described previously (Brown *et al.* 1987*b*). Plasma renin activity, plasma aldosterone and urinary aldosterone were measured by radioimmunoassays as described previously (Brown *et al.* 1987*a*), creatinine by a spectrophotometric method (Edwards & Whyte 1958) and sodium by flame photometry. The plasma volume was measured by an Evans Blue dye dilution technique (Gallery *et al.* 1979) and blood pressure using a Hawkesly random zero sphygmomanometer. Sodium excretion was expressed as its absolute 24-h excretion and as the sodium:creatinine ratio, i.e. mmol of sodium excreted per mmol creatinine excreted, to allow for any errors of urine collection. The glomerular filtration rate (GFR) was measured as the creatinine clearance (Davison & Hytten 1974), and fractional sodium excretion as the percentage of filtered sodium (plasma sodium × GFR) appearing in the final urine.

Calculations and statistics

Changes within groups were analysed by Wilcoxon's (paired) test and those between groups by the Mann–Whitney *U*-test. Differences amongst three groups were tested using analysis of variance and Scheffe's test to determine the site of significant differences ($P<0{\cdot}05$). The relations between changes in PRA and changes in sodium excretion were exponential and data were transformed to a log linear scale before determination of this regression equation. Goodness of fit was assessed by analysis of standardized residuals. Analysis of covariance was used to compare the slopes of regression lines. All data were stored on the VAX 2900 computer at the University of Nottingham, UK, and analysed using SPSS-X 2·1 statistical analyses program (Chicago, USA). Results are expressed as mean and standard deviation (SD).

Results

Both dietary groups had similar age, height, and weight at entry into the study [LS-group: age 29·0 (5·1) years, height 163 (6) cm, weight 63·6 (9·3) kg; HS-group: age 28·6 (7·5) years, height 164 (10) cm, weight 64·2 (10·0) kg]. Overall 122 women who continued to be normotensive were studied in the second trimester (60 were in the LS-group and 62 in the HS-group). Of these 122 women, 55 were studied again in the third trimester (30 LS, 25 HS) and 16 of them were studied for the third time 3 months after delivery. Unfortunately, PRA measurements were complete for only eight women in this subgroup who were studied on three occasions. Nine women (5 LS, 4 HS) were breast-feeding at the time of the postpartum study and PRA measurements were complete for all postpartum women. All the women were encouraged to participate at all stages but not all were able to do so due to constraints of time, particularly in the postpartum period, and the demands of this study.

Twenty subjects (12 LS, 8 HS), who were normotensive during the second trimester, developed PIH in the third trimester. Four of them (2 LS, 2 HS) were studied again *after* the development of PIH and these data were not analysed due to the small sample number. Only six of these 20 women (4 LS, 2 HS) developed proteinuria (>300 mg/dl) as well as hypertension and none was nephrotic. The data for proteinuric subjects were not analysed separately due to the small sample number.

Baseline (pre-study) data

Normal pregnancy. The results for the 16 women studied on three occasions are shown in Table 1. Absolute urinary sodium excretion was similar during pregnancy and postpartum, but filtered sodium was significantly higher and fractional sodium excretion significantly lower during pregnancy than at 3 months after delivery. This was true for the whole group studied and when data were analysed for only those 16 subjects who had studies at all three stages. The plasma volume and body-weight were significantly higher during pregnancy as were PRA, plasma aldosterone and urinary aldosterone excretion. The haematocrit, plasma sodium, albumin and uric acid concentrations were all decreased significantly during pregnancy (Table 1).

PIH (Table 2). The women in this group who later developed PIH had significantly higher sitting systolic blood pressure and fractional and absolute sodium excretion during their second trimester, when they were still normotensive, than the women who remained normotensive. All other pre-study variables were similar between these two groups.

Responses to changing sodium intake (Table 3)

Normal pregnancy. Low-salt diets induced falls in body-weight, plasma volume and absolute and fractional urinary sodium excretion at all stages. Glomerular filtration rate fell significantly in these women during pregnancy but not postpartum. Conversely, sodium loading resulted in an increase in body-weight, plasma volume and sodium excretion. Glomerular filtration rate increased significantly in this group during the third trimester and postpartum but not during the second trimester. Plasma sodium concentration was not influenced by these manoeuvres at any stage. Irrespective of pre-study values, PRA, plasma and urinary aldosterone were all increased significantly from baseline values by salt depletion and suppressed significantly by salt loading at all stages (Figs 1 & 2). The relation between individual changes in salt intake and the accompanying percentage change in PRA from baseline differed between pregnancy and postpartum (Fig. 3). During the second trimester this line did not pass through the origin, i.e. a rise in PRA could be expected during the week of study independent of changing salt intake. During the third trimester this line passed through the origin, as did the postpartum line, but the sensitivity (slope) of PRA response was reduced significantly from the postpartum line ($P<0{\cdot}05$).

Women who developed PIH. During the second trimester the women in this group did not achieve final urine sodium excretion as low as the continuously normotensives in the LS group or as high as the normotensives in the HS group. However, neither these final values nor the difference from baseline by day 7 were significantly different from continuously normotensive subjects. Body-weight, plasma volume and GFR all changed in a similar manner to that of normal pregnant subjects. The major abnormality within this group was the failure to stimulate plasma aldosterone significantly following salt

Table 1. Pre-study data for the 16 subjects studied at all stages

Variables	2nd trimester	3rd trimester	Postpartum
Blood pressure (mmHg)			
Systolic	99 (12)*	103 (8)	108 (8)
Diastolic	61 (8)*	65 (8)	67 (8)
Weight (kg)	60·3 (9·6)*	65·4 (10·4)*	57·0 (10·4)
Plasma volume (ml/cm)	22·0 (2·4)*	25·0 (2·4)*	18·5 (2·4)
Haematocrit (%)	34·2 (0·4)*	34·7 (0·4)*	38·7 (0·4)
Albumin (g/l)	37 (2)*	35 (2)*	45 (3)
Plasma uric acid (mmol/l)	0·22 (0·04)*	0·25 (0·04)	0·28 (0·04)
Plasma sodium (mmol/l)	137 (2)*	137 (2)*	140 (3)
GFR (ml/min)	145 (28)*	143 (36)*	99 (20)
Sodium excretion (mmol/day)	108 (24)	110 (44)	100 (36)
Na:Cr ratio	12 (2)	12 (2)	11 (3)
FENa (%)	0·38 (0·12)*	0·41 (0·20)*	0·54 (0·20)
PRA (ngAI/ml/h)	4·2 (2·4)*	5·7 (4·0)*	1·1 (0·8)
PAC (fmol/ml)	1145 (328)*	2384 (1560)*	502 (440)
Uald (nmol/day)	351 (196)*	670 (404)*	27 (12)

Results are means (SD).
*$P<0{\cdot}05$ *vs* postpartum.
Abbreviations: GFR, Glomerular filtration rate; Na:Cr, mmol of sodium/mmol creatinine excreted; FE Na, fractional sodium excretion; PRA, plasma renin activity; PAC, plasma aldosterone concentration; Uald, urinary aldosterone excretion.

Table 2. Pre-study data during the second trimester for continuously normotensive subjects (*n* = 122) and those who later developed PIH (N→PIH) (*n* = 20)

	Normal	N→PIH
Age (years)	28·8 (6·6)	27·8 (4·5)
Height (cm)	164 (11)	166 (9)
Blood pressure (mmHg)		
Systolic	103 (11)	109 (9)*
Diastolic	63 (11)	66 (9)
Weight (kg)	63·9 (8·8)	66·8 (8·6)
Plasma volume (ml/cm)	24·2 (2·2)	23·6 (2·7)
Haematocrit (%)	33·9 (2·2)	34·4 (2·7)
Albumin (g/l)	37 (2)	37 (4)
Plasma uric acid (mmol/l)	0·22 (0·11)	0·23 (0·05)
Plasma sodium (mmol/l)	137 (3)	137 (4)
GFR (ml/min)	152 (39)	145 (32)
Sodium excretion (mmol/day)	112 (40)	136 (45)*
Na:Cr ratio	12 (4)	13 (4)
FENa (%)	0·38 (0·11)	0·49 (0·23)*
PRA (ngAI/ml/h)	4·1 (3·3)	4·9 (2·7)
PAC (fmol/ml)	1489 (1122)	1489 (738)
Uald (nmol/day)	418 (429)	359 (180)

Results are means (SD).
*$P<0{\cdot}05$.
For list of abbreviations see Table 1.

depletion though PRA, plasma and urinary aldosterone otherwise behaved similarly to the pattern seen in women who remained normotensive (Fig. 4). The relation between change in salt intake and change in PRA in the second trimester in these women resembled that seen in the third trimester in the women who remained normotensive (Fig. 3).

Table 3. Variables related to sodium excretion before (day 0) and after (day 7) dietary sodium manipulations

	Day	(*n*)	Weight (kg)	Plasma volume (ml/cm)	Plasma Na (mmol/l)	GFR (ml/min)	Na excretion (mmol/l)	Na:Cr	FENa (%)
Normal subjects									
Low-salt									
2nd trimester	0	(60)	63·6 (8·5)	24·3 (3·1)	137 (2)	159 (43)	112 (50)	12 (5)	0·36 (0·15)
	7		62·5*** (7·7)	23·4*** (3·1)	137 (2)	137*** (39)	27*** (12)	3*** (2)	0·11*** (0·08)
3rd trimester	0	(30)	67·9 (10·4)	25·7 (2·8)	137 (2)	151 (44)	108 (50)	12 (5)	0·36 (0·16)
	7		66·7*** (10·4)	25·1* (2·8)	137 (2)	132** (33)	28*** (16)	3*** (2)	0·11*** (0·06)
Postpartum	0	(9)	60·0 (12·3)	19·2 (2·7)	139 (3)	107 (21)	104 (39)	12 (4)	0·56 (0·18)
	7		58·8** (12·6)	18·0 (3·3)	140 (2)	101 (18)	14*** (12)	2*** (2)	0·08*** (0·06)
High-salt									
2nd trimester	0	(62)	64·2 (9·4)	24·1 (2·4)	137 (2)	143 (40)	114 (47)	12 (5)	0·39 (0·16)
	7		65·5*** (8·7)	25·4*** (3·2)	137 (2)	146 (40)	256*** (68)	29*** (7)	0·96*** (0·47)
3rd trimester	0	(25)	67·0 (10)	25·6 (3·0)	137 (3)	135 (25)	97 (35)	11 (5)	0·38 (0·15)
	7		68·7*** (10)	26·3* (3·0)	137 (2)	147* (25)	257*** (83)	28*** (9)	0·94***(0·30)
Postpartum	0	(7)	53·3 (5·8)	17·6 (1·8)	141 (3)	88 (18)	95 (44)	12 (7)	0·51 (0·23)
	7		53·6* (5·8)	19·2 (2·6)	140 (2)	103* (18)	251*** (78)	31*** (7)	1·21*** (0·26)
N→PIH									
Low-salt									
2nd trimester	0	(12)	67·4 (6·2)	24·2 (2·5)	138 (2)	153 (31)	144 (67)	14 (4)	0·46 (0·21)
	7		66·0*** (6·2)	22·6* (1·8)	136 (2)	138* (28)	36*** (19)	4*** (2)	0·15*** (0·11)
High-salt									
2nd trimester	0	(8)	65·9 (11·6)	22·8 (3·4)	136 (3)	136 (31)	125 (70)	13 (5)	0·52 (0·20)
	7		67·5** (11·2)	24·8* (3·9)	137 (2)	133 (40)	237*** (71)	28*** (6)	0·87** (0·22)

Results are means (SD).

*$P<0·05$, **$P<0·01$; ***$P<0·001$ day 0 *vs* day 7.

For abbreviations see Table 1.

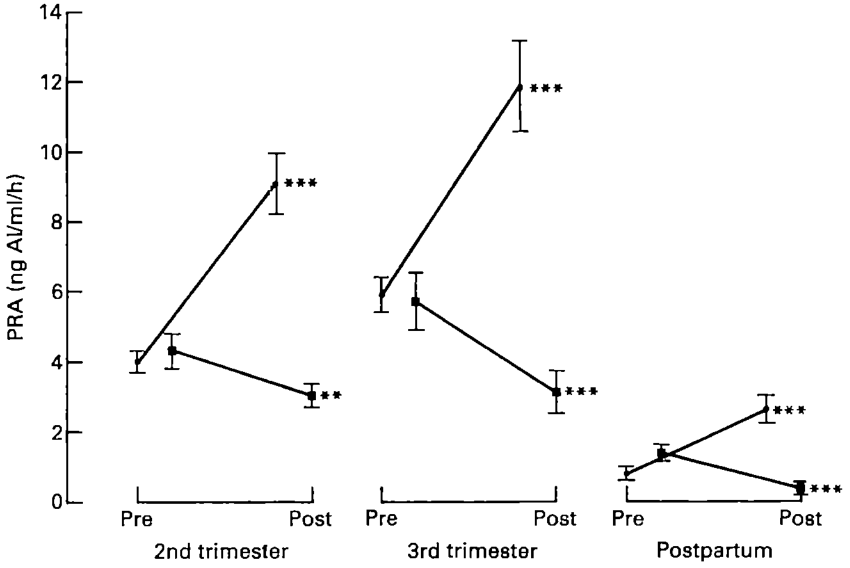

Fig. 1. Plasma renin activity (PRA) before and after low (●) and high (■) salt diets. $^{**}P<0{\cdot}01$; $^{***}P<0{\cdot}001$.

Discussion

These data demonstrate that although the components of the renin–aldosterone system respond appropriately around their new 'set-points' during pregnancy, the sensitivity of this system to changes in salt intake is reduced compared to postpartum. Furthermore, women who later develop pregnancy-induced hypertension do not stimulate plasma aldosterone concentration significantly following salt depletion during mid-pregnancy though plasma renin activity and urine aldosterone excretion rise appropriately. These, and several other points raised by these data, are discussed below.

Normal pregnancy

It is clear from the baseline (pre-study) data that 24-h urinary sodium excretion during *ad libitum* eating is not significantly altered by pregnancy, despite increments in plasma volume and glomerular filtration rate, both factors which tend to enhance sodium excretion. The concurrent stimulation of the renin–aldosterone system suggests a significant role for this system in balancing these sodium-losing stimuli though it is evident from Fig. 3 that, particularly during mid-pregnancy, there is a 'sodium-independent' component of the R–A system, as discussed in detail previously (Brown *et al.* 1987*a*).

Against this 'steady-state' background it is then apparent that the R–A system alters in a manner which allows excretion or retention of sodium to help maintain a fairly constant plasma volume when salt intake is changed acutely (Figs 1 & 2). However, these changes in salt intake were accompanied by significant changes in body-weight as well as in sodium excretion. Acute changes in body-weight signify alterations to the extracellular fluid volume (Walser 1985). As plasma volume altered little following changes in salt intake, 'buffering' of sodium from and into the interstitial fluid space must have occurred as a mechanism to help maintain a constant intravascular volume during pregnancy. This is the first report of this phenomenon in pregnancy, and while direct measurements of the extracellular fluid volume will be required to confirm it, the same mechanisms are known to operate in non-pregnant subjects (Gauer & Henry 1976). Close examination of Table 3 suggests that these mechanisms may not be as 'finely tuned' during pregnancy as after delivery. Pregnant women did not reduce sodium excretion as far as expected following their 20 mmol sodium intake and had final 24-h sodium excretions 7 or 8 mmol above this level. When consideration is given to the expected accumulation of about 3–4 mmol sodium per day during pregnancy (Chesley 1978) these differences become even greater. These were not strict metabolic balance studies nor truly longitudinal for all subjects and therefore dogmatic statements about these find-

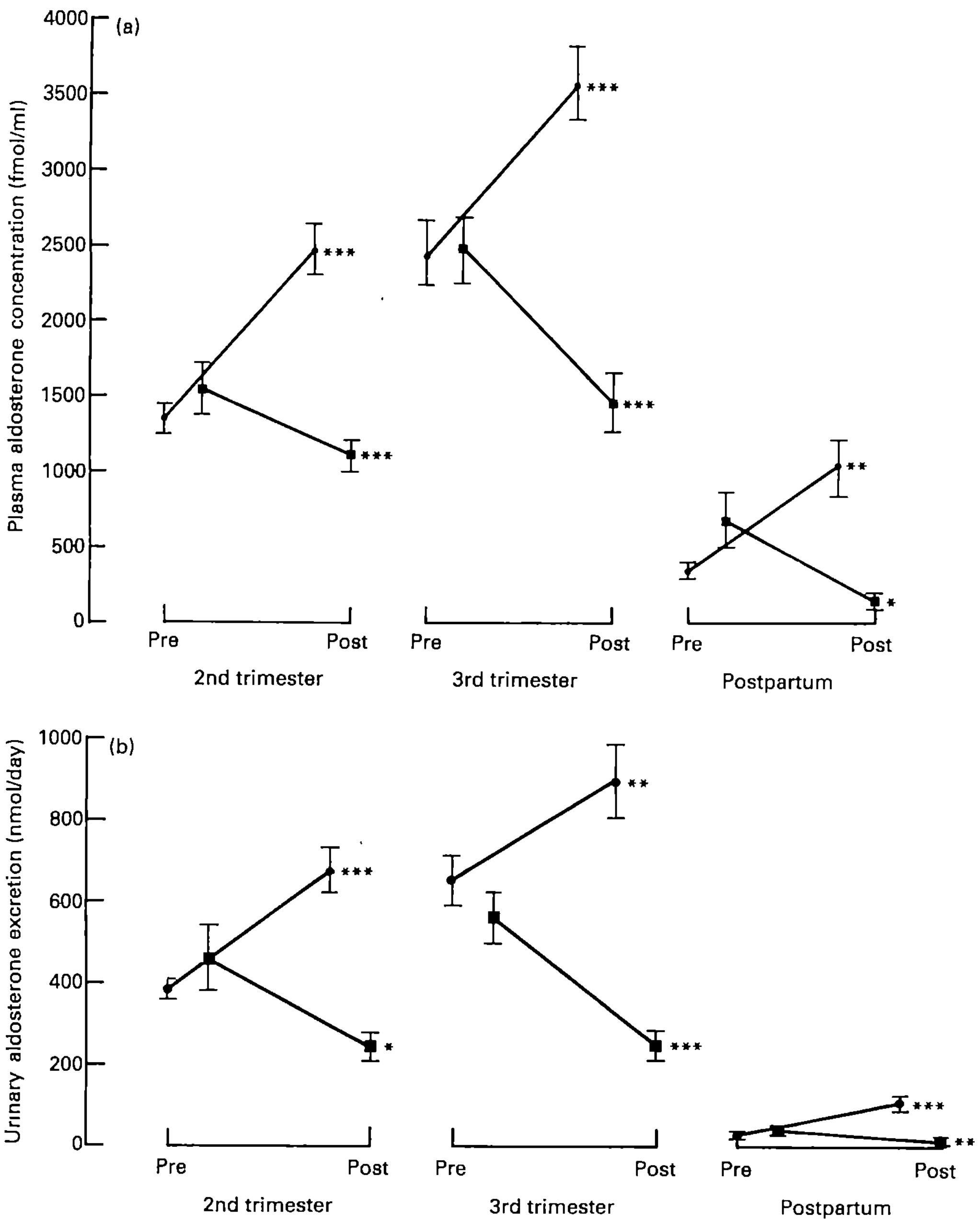

Fig. 2. Plasma aldosterone concentration (a) and urinary aldosterone excretion (b) before and after low (●) and high (■) salt diets. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

ings cannot be made but it would seem that there is greater difficulty adapting to sodium depletion during pregnancy than after delivery. Furthermore, postpartum subjects, some of whom were breast-feeding, achieved urinary sodium excretions below the 20 mmol limit, an appropriate finding when sodium losses in breast milk (not measured directly here) are accounted for. In addition, weight loss was almost identical following low-salt diets during pregnancy and postpartum. When consideration is given to the fact that pregnant women should have been *gaining*

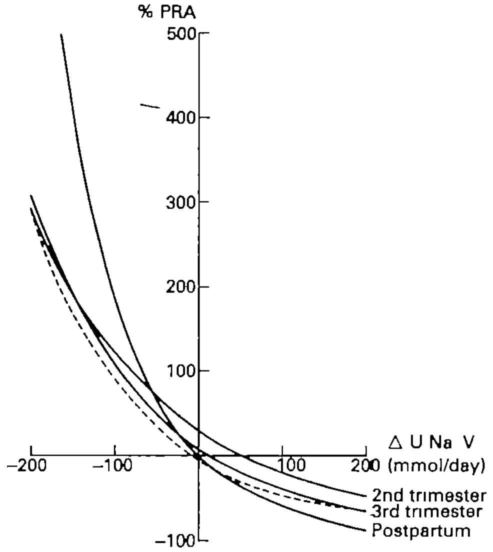

Fig. 3. Percentage change in plasma renin activity from baseline (% PRA) (y) related to change in sodium excretion (U Na.V) (x). (———, Normal; -----, N→PIH (2nd trimester) These equations are:

2nd trimester $y = 112\ e^{-0\cdot006x} - 83$ ($r = -0\cdot72$, $P<0\cdot001$)

3rd trimester: $y = 98\ e^{-0\cdot007x} - 89$ ($r = -0\cdot74$, $P<0\cdot001$)

Postpartum: $y = 94\ e^{-0\cdot011x} - 92$ ($r = -0\cdot79$, $P<0\cdot001$)

N→PIH: $y = 78\ e^{-0\cdot008x} - 83$ ($r = -0\cdot85$, $P<0\cdot001$)

weight (approximately 0·4 kg/week) (Hytten & Leitch 1964) at the times of these studies, it is likely that these equivalent reductions in bodyweight did reflect greater sodium losses during pregnancy. Similarly, Sarles *et al.* (1968) found that pregnant women could not achieve sodium balance on a diet of 50 mmol sodium per day, and Lindheimer (1980) reinterpreted the studies of Bay & Ferris (1979) to demonstrate effective sodium loss in those fed a low-salt intake during pregnancy.

The difference in the sensitivity of PRA response to changing salt intake between pregnancy and postpartum (Fig. 3) is relevant to these findings. Figure 3 demonstrates that once salt depletion of more than 50 mmol per day occurred, a greater percentage increase in PRA resulted postpartum than during either stage of pregnancy. It may be argued that this was the effect of breast-feeding in the postpartum group which resulted in greater renin release to help replace extrarenal sodium losses. If this is so, then this is precisely the response expected in this group of pregnant women who, as Lindheimer (1980) has stressed, should also be retaining extra sodium to meet both maternal and fetal sodium needs. Some caution must be placed on these findings at present in view of the largely cross-sectional nature of this study (when comparing pregnant and postpartum data) but this interpretation of these data is certainly in keeping with the failure to lower final urinary sodium excretion as effectively during pregnancy as postpartum following the same low-salt diet.

Pregnancy-induced hypertension

Although women who later developed PIH had higher baseline sodium excretion than continuously normotensive subjects during mid-pregnancy, it is unlikely that this reflected a physiologically significant sodium-losing tendency in this group as plasma volumes were similar to those in continuously normotensive subjects. Furthermore, following sodium depletion, weight loss was slightly greater in those who later developed PIH, a finding appropriate to the greater fall in urine sodium excretion from this higher baseline in these women. However, like continuously normotensive subjects, these women failed to achieve the low urinary sodium excretion seen after low-salt diets in postpartum studies. These findings suggest that the *short-term* ability to adapt to changes in salt intake is intact during mid-pregnancy in women who later develop PIH, at least to the same extent as continuously normotensive women.

However, major abnormalities occurred in the renin–aldosterone system before the clinical development of PIH. Although baseline renin and aldosterone measurements during mid-pregnancy were similar in this group to those in continuously normotensive subjects, as reported previously (Gallery *et al.* 1980), there was a significant failure to increase plasma aldosterone to the same levels as normotensive subjects after sodium depletion at this stage. This could not be explained by hypokalaemia or hypernatraemia and has been reported previously following a diuretic challenge after delivery in women with a previous history of PIH (Al-Khader *et al.* 1980). As both groups of pregnant women appeared to have similar overall sodium excretion following these dietary manoeuvres, the significance of this finding is unclear. Certainly there was dis-

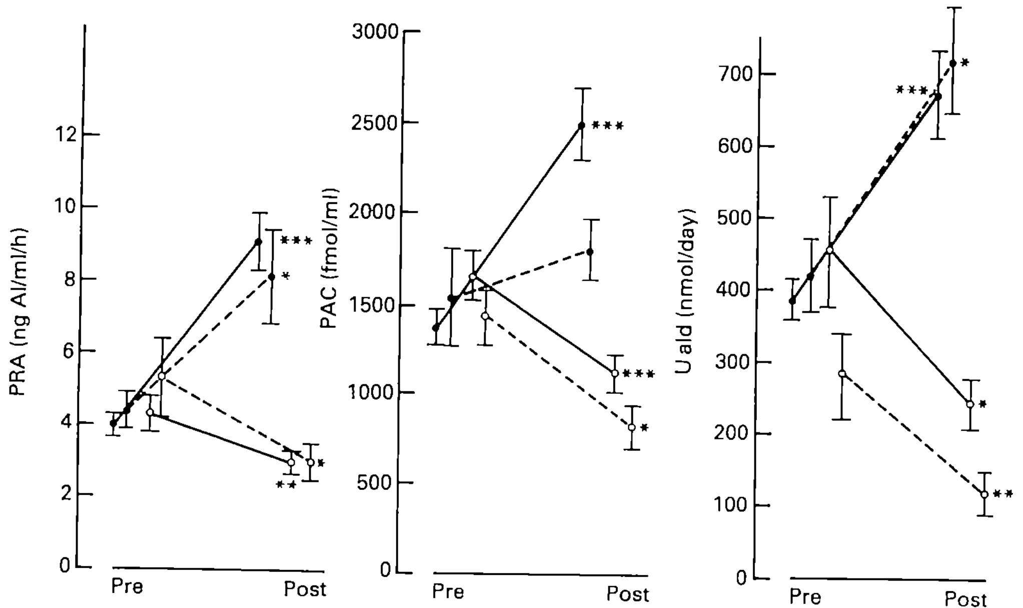

Fig. 4. Plasma renin activity (PRA), plasma aldosterone concentration (PAC) and urine aldosterone excretion (Uald) before and after low (●) and high (○) salt diets in continuously normotensive subjects (———) and in those who later developed pregnancy-induced hypertension (——). $^{*}P<0{\cdot}05$; $^{**}P<0{\cdot}01$; $^{***}P<0{\cdot}001$.

sociation between renin and aldosterone in response to salt depletion in women who later developed PIH, and unless the sensitivity to aldosterone was altered in these women, this should have led to a greater difficulty adapting to salt depletion than in continuously normotensive subjects. However, as this did not occur, it is probable that other factors altered to retain sufficient sodium by the end of 1 week in this group. The implication of these findings throughout the whole of pregnancy was not addressed by this study but it is conceivable that sodium loss, and hence plasma volume reduction, could occur in some of these pregnant women if adequate salt intake occurred for the duration of pregnancy. This remains speculative at present but should be addressed in future studies.

Urinary aldosterone did not follow the same pattern as plasma aldosterone concentration following salt depletion in women who later developed PIH, but these findings are not necessarily incompatible and may be due to: (a) altered aldosterone metabolite excretion, known to occur in normal pregnancy (Watanabe *et al.* 1963; Coghlan & Blair-West 1967) but unaddressed in those with PIH; (b) failure of the normal reabsorption of most filtered aldosterone by the distal nephron (Luetscher *et al.* 1965) resulting in 'normal' urinary aldosterone values despite submaximal plasma values; and (c) excretion of another substance interfering with the urinary aldosterone assay in these subjects after salt depletion.

Reasons for failure to stimulate aldosterone adequately after salt depletion were not addressed by this study. It is possible that renin, which was stimulated appropriately, did not result in angiotensin II production, as described in a group of non-pregnant hypertensive subjects (Sambhi *et al.* 1976). It may be that other factors, e.g. ACTH, are important mediators of aldosterone release in pregnancy and are deficient in those who develop PIH. Finally, aldosterone secretory rate may already be maximal in these women, enabling this hormone to be suppressed but not further stimulated. Further research will clarify these issues.

In conclusion, these studies suggest that normal women have slightly greater difficulty adapting to salt depletion during pregnancy than after delivery, possibly due to a 'blunted' response of the renin–aldosterone system. Those women destined to develop PIH are still able to stimulate PRA but not plasma aldosterone, following salt depletion during mid-pregnancy. Although this does not greatly affect *short-term* sodium regulation in these women, it is conceivable that over a longer time course salt-wasting could ensue if an adequate salt intake is not provided. Such conclusions must be tempered by the fact that these studies, although the largest conducted to date, were not performed in a metabolic ward, but should provide the stimulus for further research.

Meanwhile, the pregnant woman remains aware of conflicting public debate about the desirability of certain salt intakes. Based upon our findings, we would not recommend a reduction in salt intake during pregnancy from that which was being eaten before conception.

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Can ultrasound reliably diagnose ectopic pregnancy?

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Summary. A total of 252 women with amenorrhoea and with abdominal pain or vaginal bleeding, or both, had an emergency high-resolution ultrasound sector scan. In 100 women the symptoms were unrelated to any identifiable abnormal ultrasound finding, none of them was pregnant and their symptoms settled spontaneously; 33 other women had follicular or luteal cysts and 30 had pelvic inflammatory disease. Histological examination confirmed an ectopic pregnancy in 60 women (24%); in seven a live fetus was observed outside the uterus allowing a confident diagnosis of ectopic pregnancy; in 27 the thickness of the endometrium was >10 mm (sensitivity 50%, specificity 84%, positive predictive value 28%, negative predictive value 87%); in 15 the uterine area measurement was <20 cm^2 (sensitivity 72%, specificity 41%, positive predictive value 20%, negative predictive value 79%); and 43 had an adnexal mass volume >10 ml separate from the ovary (sensitivity 85%, specificity 37%, positive predictive value 23%, negative predictive value 90%). Only three had negative ultrasound findings. The negative predictive value of an ultrasound examination could be increased to 96% by using a combination of these ultrasound features. The addition of hCG (>25 i.u./l) improved the specificity to 98% and the negative predictive value to 100%. These criteria may improve the ultrasound diagnosis of ectopic pregnancy.

The true incidence of ectopic pregnancy is difficult to determine, but is considered to be increasing worldwide (Rubin *et al.* 1983). Despite the introduction of modern diagnostic methods such as ultrasonography and rapid and specific hCG assays which lead to early surgical intervention, ectopic gestation remains the major cause of maternal mortality in the first trimester of pregnancy (Dorfman 1983; Report on Confidential Enquiries into Maternal Deaths in England and Wales 1979–1981 (1986)). The death rate from ectopic pregnancy is approximately 1 in 1000 cases, delay in making the diagnosis being the major underlying reason for this (Sanders 1978; Hazekamp 1980).

Traditionally, the primary role of ultrasound is limited to excluding this life-threatening condition by the demonstration of an intrauterine pregnancy (Bradley *et al.* 1983). In a prospective investigation of real-time ultrasonography as the sole diagnostic test in suspected ectopic pregnancy, an accurate diagnosis of the presence or absence of this disease was made in 94% of 32 women in whom this condition was suspected (Gleicher *et al.* 1983).

This study was initiated to investigate the

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diagnostic value of high-resolution abdominal sector scanning as the sole investigation in the diagnosis and exclusion of ectopic pregnancy.

Patients and methods

During an 18-month period (from January 1985) 252 women believed to be pregnant, were recruited consecutively into this prospective study. All women presented with either abdominal pain, vaginal bleeding, or both, and were referred to the emergency gynaecological ultrasound department at King's College Hospital with a provisional diagnosis of suspected ectopic pregnancy. After informed consent, comprehensive clinical details were recorded on all patients.

The examinations were performed with the full bladder technique using a Diasonics DRF 100 sector scanner with a 3-MHz transducer. All the scans were performed by one of the authors (I.S.) within 24 h of admission to the casualty department or gynaecology ward. The ultrasonographer was unaware of the result of any other biochemical investigations at the time of the scan.

During the ultrasound examination the operator looked for any features suggestive of an intrauterine pregnancy, including a gestational sac and live fetus. If pregnancy was not confirmed, the following ultrasound features were investigated.

(1) The presence and size of any adnexal mass. The adnexal mass volume was calculated according to the formula for the volume of an ellipsoid:

$$V = \frac{4\pi abc}{3}$$

where V is the volume, $\pi = 3{\cdot}14$ and abc are the three radii.

Receiver operator characteristic curves (Swets 1964; Richardson *et al.* 1985) were constructed by comparing the sensitivity (true-positive rate) and false-positive rates at varying cut-off points, e.g. adnexal mass volume levels of 5, 10 and 15 ml. An adnexal mass volume >10 ml, separate from the ovary, had the highest sensitivity and was considered abnormal.

(2) Free fluid or blood clots in the pouch of Douglas.

(3) The maximal uterine area. This was assessed by obtaining the maximal transverse section of the uterus and encircling the uterine outline with online calipers. The measurements were made three times by the same observer and accepted if they were within 2 cm^2 of each other. Receiver operator curves for true-positive and false-positive rates were constructed at cut-off values of 10, 15 and 25 cm^2. A uterine area measurement <20 cm^2 was defined as abnormal for this study.

(4) The endometrial thickness. This was assessed by obtaining a longitudinal section of the uterus and measuring the outer to outer thickness of the endometrium. Receiver operator curves were constructed at cut-off values of 5, 8, 10, and 15 mm. An endometrial thickness >10 mm was considered abnormal.

At the time of each ultrasound examination a serum blood sample was taken and the serum analysed subsequently for β-hCG, using a commercially available kit (Amerlex M hCG RIA, Amersham).

All the patients were followed up after the ultrasound examination to ascertain the clinical outcome.

Statistical methods

Receiver operator characteristic curves (Richardson *et al.* 1985) were constructed to select cut-off values for three of the ultrasound indices analysed: maximal uterine area, endometrial thickness and volume of adnexal mass. By plotting the true-positive rate (sensitivity) against the false-positive rate (1−specificity) at different cut-off levels, it is possible to select an appropriate cut-off value for each of the ultrasound variables measured. As the aim of this study was to investigate the value of abdominal ultrasound in the management of suspected ectopic pregnancy, we chose cut-off levels which maximized sensitivity while accepting high false-positive rates.

Differences in the mode of presentation and clinical characteristics of women with ectopic pregnancy and other conditions were analysed using the χ^2-test and one-way analysis of variance.

Results

The final diagnosis and clinical characteristics of the 252 women studied are summarized in Table 1. There were no apparent differences in the mean age, race, past obstetric history, previous medical or surgical history or previous contraceptive usage between the different diagnostic subgroups. Significantly more women

Table 1. Mode of presentation, clinical characteristics and outcome in 252 women with suspected ectopic pregnancy

								Mode of presentation									
	Total		Age (years)		Race*			Abdominal pain		Irregular bleeding		Adnexal mass		Pelvic tenderness		Body temperature (°C)	
Clinical outcome	*n*	(%)	Mean	(SD)		*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	Mean	(SD)
Ectopic pregnancy	60	(24)	27	(4·7)	W B A	46 10 4	(77) (17) (7)	56	(93)	49	(81)	2	(3)	46	(77)	37	(0·6)
Normal intrauterine pregnancy	25	(10)	24	(6)	W B A	18 6 1	(72) (24) (4)	20	(80)	9	(36)	1	(4)	16	(64)	37	(0·5)
Incomplete abortion	3	(1)															
Missed abortion	1	(0·4)															
Pelvic inflammatory disease	30	(12)	22	(4)	W B A	19 10 1	(63) (33) (3)	30	(100)	10	(33)	3	(10)	23	(77)	37	(1)
Ovarian cyst	33	(13)	26	(7)	W B A	25 6 2	(76) (18) (6)	25	(76)	21	(64)	4	(12)	26	(79)	36	(0·05)
No abnormality detected	100	(40)	27	(5·2)	W B A	66 28 6	(66) (28) (6)	84	(84)	63	(63)	3	(3)	74	(74)	37	(0·7)
			NS			NS		*P*<0·05*		*P*<0·001*		NS		NS		NS	

* Abbreviations for race: W, White caucasian; B, black African; A, Asian.

There were no significant differences between any of the different clinical outcome subgroups. Significantly more women in the ectopic pregnancy group had abdominal pain ($P<0{\cdot}05$) and irregular bleeding ($P<0{\cdot}001$) compared with the normal intrauterine pregnancy group.

Table 2. Test performance of the ultrasound findings in the diagnosis of ectopic pregnancy

Test finding	Sensitivity (%)	Specificity (%)	Predictive value of positive test (%)	Predictive value of negative test (%)
Adnexal ring structure with fetal pole	100	100	100	100
Free fluid in pouch of Douglas	55	84	41	87
Endometrial thickness >10 mm	50	84	28	84
Uterine area <20 cm²	72	41	20	79
Adnexal mass >10 ml	85	37	23	90
Any abnormality	65	57	25	87

in the ectopic pregnancy subgroup had abdominal pain ($P<0{\cdot}05$) and irregular vaginal bleeding ($P<0{\cdot}001$) than those with intrauterine pregnancies which continued uneventfully.

Table 2 shows the ultrasound findings in the 60 women with ectopic pregnancy, analysed in relation to the diagnostic accuracy of the particular ultrasound feature investigated. Apart from the finding of an adnexal ring structure with a live fetal pole, none of the variables measured had a positive predictive value >41%. Similarly, the sensitivity (50–85%) or specificity (37–84%) of these measurements taken singly was too low to be of practical clinical value. The predictive value of a negative result, that is the absence of any abnormal ultrasound finding, was 79–90%.

Figure 1 shows the predictive value of various combinations of the abnormal ultrasound findings. The combination of endometrial thickness and uterine area measurements gave the best result with a sensitivity of 86% and a specificity of 82%. Adding a third ultrasound feature (adnexal mass volume) did not improve the test performance.

Circulating hCG was detected (>25 i.u./l) in all 60 women with ectopic pregnancy, 81% were <10th centile of the range in normal pregnancy (Grudzinskas *et al.* 1988). Table 3 summarizes the predictive value of hCG and an abnormal ultrasound finding either alone or in combination. The sensitivity and positive predictive value of a positive test result for hCG (>25 i.u./l) was considerably improved (sensitivity from 81·3 to 98% and positive predictive value from 62·5 to 95%) by combination with any abnormal ultrasound finding.

Discussion

The aim of this study was to assess the usefulness of a single ultrasound examination in the differential diagnosis of abdominal pain in women of reproductive age in whom the diagnosis of ectopic pregnancy is suspected. At present the diagnostic accuracy of ultrasound is not considered reliable except in the few patients with an intact ectopic gestational sac containing a live fetus. The results of our study would support this conclusion. It is noteworthy that a live extra-uterine fetal pole was noted in seven of our 60 women (12%) who had a proven ectopic pregnancy. This proportion is higher than that reported by others (Robinson & De Crespigny 1983), and may be a reflection of patient selection in that this ultrasound clinic actively encouraged referral of women with suspected ectopic pregnancy.

All the examinations reported here were made by a single operator who was unaware of

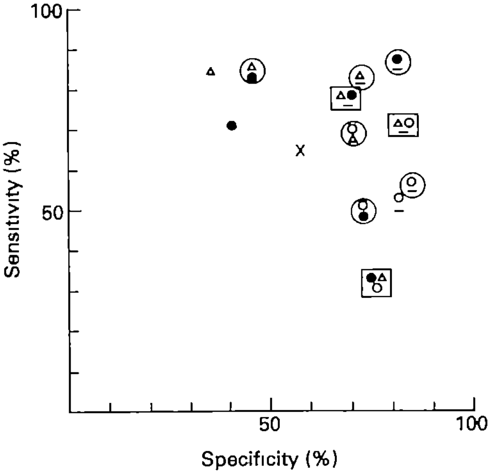

Fig. 1. Predictive value of ultrasound findings taken singly and in combination. ×, Any abnormality; ○, free fluid in the pouch of Douglas; ●, uterine area <20 cm²; △, adnexal mass volume >10 ml, —, endometrial thickness >10 mm.

Table 3. Test performance of the ultrasound findings and serum hCG used alone or in combination in the diagnosis of ectopic pregnancy

Test finding	Sensitivity (%)	Specificity (%)	Predictive value of positive test (%)	Predictive value of negative test (%)
hCG>25 i.u./l	100	81	63	100
Any abnormal ultrasound finding	65	57	25	87
Adnexal ring structure with a live fetal pole	100	100	100	100
hCG>25 i.u./l and any abnormal ultrasound finding	95	98	95	100

the result of the β-hCG test since the intention of the study was to assess the value of ultrasound alone in the diagnosis or exclusion of ectopic pregnancy. Two of the four ultrasound features chosen were entirely objective measurements, the cut-off levels being chosen by reference to receiver operator characteristics. The presence and size of an adnexal mass as well as free fluid in the pouch of Douglas were subjective assessments and hence open to the criticism of operator skill and experience. All seven women with an intact live ectopic pregnancy had a pseudogestational sac within the uterus, 50% of the remaining patients with ectopic pregnancy had a thickened endometrium (>10 mm). This feature may reflect the hormonal environment and is probably an even earlier feature than a decidual cast.

An early intrauterine pregnancy can first be detected as a ring-shaped structure within the uterus from 4·5 weeks amenorrhoea. A pseudo ring may be seen with ectopic pregnancy and can be distinguished from a true gestational sac by its bright asymmetrical echogenic border, eccentric position within the uterus and double-ring appearance (Bradley *et al.* 1983). The major difficulty, however, lies in distinguishing an ectopic gestation without a pseudogestational sac from an early normal or failed intrauterine pregnancy where no gestational sac can be seen by ultrasound. The negative predictive value of a single ultrasound examination could be improved by using a combination of free fluid and adnexal mass (88%), endometrial thickness and free fluid (94%), uterine area and adnexal mass (95%), endometrial thickness and adnexal mass (95%), endometrial thickness and uterine area (96%), free fluid and uterine area (96%). Adding a third variable did not significantly alter the predictive value of negative test results. The combination of endometrial thickness and uterine area improved the positive predictive value to 55%. In summary, this investigation has demonstrated that in the absence of the ultrasound features described, there is a 96% probability that there is no ectopic pregnancy.

Although the criteria described here may improve the value of an ultrasound examination for excluding ectopic pregnancy when there is clinical suspicion, the sole use of an ultrasound examination will not reliably diagnose this life-threatening condition. When any ultrasound abnormality is found in combination with detectable hCG (>25 i.u./l), the diagnostic efficacy for ectopic pregnancy is marginally improved (see Table 3). This would suggest that when choosing between ultrasound and a biochemical test to investigate a clinically stable woman with a suspected ectopic pregnancy, the decision should depend on the facilities available in the given institution. In particular, it should be noted that high-resolution ultrasound is a time-consuming, operator-dependent procedure which does not give significantly better results than hCG measurements alone. Although complete filling of the bladder was always achieved in the patients studied, it is considered, not least by the patients, a disadvantage of the technique. It is in this respect that vaginal ultrasonography should improve patient compliance. The results of prospective studies in combination with quantitative hCG estimations are awaited.

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Demonstration of ectopic pregnancy by transvaginal ultrasound

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Summary. Transvaginal ultrasound was used in the assessment of 148 patients clinically suspected of having an ectopic pregnancy. Transvaginal ultrasound allowed earlier demonstration of an intrauterine pregnancy without confusion with a pseudosac. Of the 36 patients with an ectopic pregnancy, this was strongly suspected in 29 (81%). A live extrauterine fetus was seen in 8 (22%), ectopic trophoblast or a gestation-sac-like structure in 19 (53%), and a pelvic haematoma in 2 (6%). Transvaginal ultrasound is advocated as the technique of choice in patients suspected of having an ectopic pregnancy.

Although diagnostic ultrasound has played an increasing role recently in the assessment of patients suspected of having ectopic pregnancy, its major place has been in excluding an intrauterine gestation in patients confirmed to be pregnant. Its use as a specific investigation to demonstrate the ectopic pregnancy has been disappointing. Although Gleicher *et al.* (1983) in a small study were able to see the 'gestation sac' in the adnexa in 8 of 9 patients with an ectopic pregnancy, other authors reporting larger series have been much less successful. Mahony *et al.* (1985) described abnormal adnexal findings in 80% of patients with an ectopic pregnancy but many of these were non-specific and commonly present in other conditions or indeed in normal patients, e.g. free intraperitoneal fluid. They were able to see the ectopic gestation sac in only 5 of 35 patients who subsequently proved to have an ectopic pregnancy.

When ultrasound findings are correlated with hormone tests, valuable clinical information is obtained in assessing women suspected of having an ectopic pregnancy (Robinson *et al.* 1985; Kadar & Romero 1982). However, because of the difficulty in determining the appropriate timing of surgery in patients with a positive pregnancy test and empty uterus on ultrasound, some authors have advocated the use of a 'discriminatory zone' (Romero *et al.* 1985; Nyberg *et al.* 1987). Unfortunately only some 41% of patients have a serum human chorionic gonadotrophin (hCG) level above this zone when they first present (Romero *et al.* 1985). If the discriminatory zone is to be used each laboratory must develop its own range, but this is unlikely to occur especially in some of the smaller units. In addition, patients with a recent complete abortion will continue to present a diagnostic dilemma as even the urinary pregnancy test, the random urinary hCG, may remain positive for up to 3 weeks after termination of pregnancy (Van der Lugt & Drogendijk 1985).

Despite the contribution made by transabdominal diagnostic ultrasound in the management of patients suspected of having an ectopic pregnancy, far greater assistance could be provided to the clinician were the ectopic pregnancy able to be demonstrated in the adnexa with more reliability. This paper investigates whether the improved images provided by transvaginal ultrasound provide a more accurate assessment of adnexal pathology.

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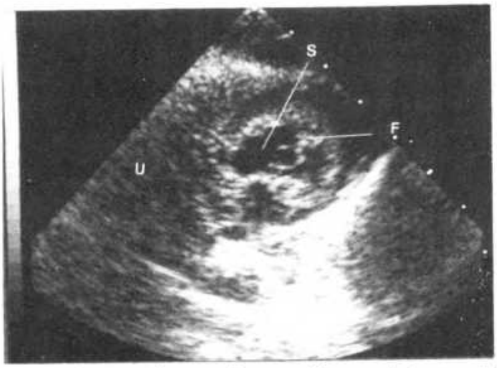

Fig. 1. An ectopic gestation sac (S) containing a yolk sac and a fetus (F) in which fetal heart movements could be demonstrated. U = uterus.

Patients and methods

Between June 1986 and August 1987, 152 consecutive patients were referred for an ultrasound examination with a clinical suspicion of an ectopic pregnancy. A General Electric RT 3600 scanner with a 5-Mz phased array vaginal transducer was used to examine all patients except those in whom a live intrauterine pregnancy was demonstrated transabdominally. In all patients, B-mode scanning alone was used. Four patients in whom clinical follow-up could not be obtained were excluded, leaving a study group of 148 patients.

When a structure was suspected to be a gestation sac, whether intrauterine or ectopic, a careful search of its contents for fetal heart movements was made (Fig. 1). If these were not found, it was identified as a gestation sac when a fluid-filled area was surrounded by an echogenic rim of presumed trophoblast (Fig. 2). In addition, a yolk sac was looked for within the sac. A pelvic haematocele was diagnosed when a clearly defined relatively echogenic lesion was identified within the pelvis separate from the uterus and ovaries. When the haematocele contained a localized echogenic area within it but no gestation sac this was diagnosed as ectopic trophoblast (Fig. 3). The presence or absence of free fluid within the pelvis, usually echo free and taking the shape of surrounding structures, was not included as a diagnostic feature.

Results

Of the 148 patients, 72 were found to have an intrauterine pregnancy. In 51 patients a definitive diagnosis of an intrauterine pregnancy was made as fetal heart movements could be seen within the gestation sac, while in 21 patients a gestation sac alone was visible in the uterus although on occasions a yolk-sac-like structure was seen in the absence of fetal heart movements. Pregnancies as early as 4·5 weeks could be demonstrated (de Crespigny *et al.* 1988). There was no patient in whom an intrauterine echo-free area or 'pseudosac' was confused with a gestation sac. A vaginal scan was performed in all patients in whom fetal heart movements could not be seen on abdominal ultrasound as this allowed both demonstration of fetal heart movements in many of the small gestation sacs when these could not be seen transabdominally, and also the diagnosis of five intrauterine gestation sacs of 4–5 weeks size which could not be seen abdominally.

Of the 36 patients with an ectopic pregnancy, the diagnosis was suggested by the presence of an ectopic gestation sac or pelvic haematocele in 29 (81%). A live fetus was demonstrable in a gestation sac outside the uterus in 8 patients (22%). In a further 19 patients (53%) a gestation-sac-like structure (14 patients) or trophoblast surrounded by blood clot (5 patients) was visible in the adnexa, although no fetal heart movements were demonstrable. In two (6%) of the patients with an ectopic pregnancy, a pelvic haematoma was diagnosed in the absence of a visible extrauterine pregnancy. There were two false-positives, in one patient an adnexal gestation sac was suspected but not found at laparoscopy; in another, an ectopic pregnancy was suspected because of the presence of a large pelvic haematoma but no gestation sac or trophoblast on ultrasound, which proved to have resulted from a ruptured ovarian

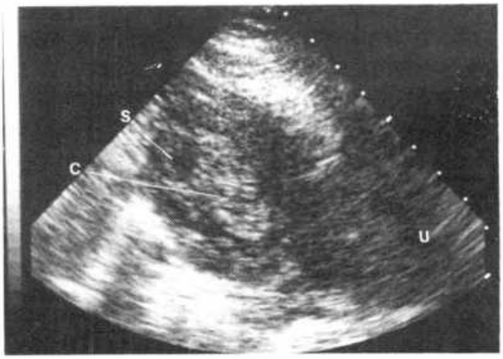

Fig. 2. Ectopic gestation sac (S) containing no demonstrable fetus. The echogenic chorion (C) around the sac is clearly visible.

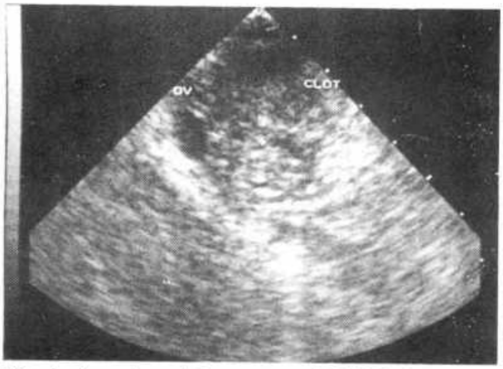

Fig. 3. An adnexal haematoma (CLOT) is present which contains a localized echogenic area behind it which is typical of trophoblast (between the markers—ECT). No gestation sac was visible. The ovary (OV) is seen laterally.

cyst. Free intraperitoneal fluid within the pelvis was not used as a diagnostic criterion because of the frequency with which it was present in other normal patients, although free fluid was present in the flanks in two patients, both of whom had an ectopic pregnancy.

There were seven patients with an ectopic pregnancy in whom the diagnosis was not suspected on ultrasound. All were between 4 and 8 weeks amenorrhoea. Five of the patients were laparoscoped immediately because ultrasound demonstrated an empty uterus and they had hormonal evidence of pregnancy. The other two were laparoscoped 6 and 9 days after ultrasound because of persistent pain.

Of the 45 patients with a normal pelvis on ultrasound, this was confirmed in 35 (77%). Eight of the 35 had a laparoscopy with normal findings whilst the remainder settled without treatment, seven of whom were felt to have had a recent miscarriage. Three patients (7%) with a normal ultrasound examination were subsequently proven to be pregnant despite the absence of any pregnancy on ultrasound but all were less than 4·5 weeks gestation at the time of the examination. In 7 of the 45 patients (16%) an ectopic pregnancy was 'missed' on ultrasound. These results are summarized in Table 1.

Discussion

The improved views provided with transvaginal ultrasound allowed a diagnosis of an ectopic pregnancy to be made using ultrasound alone in 81% of affected patients, the pregnancy being actually demonstrated in 75%. An adnexal gestation sac with its trophoblast ring and often containing a yolk sac or live fetus should seldom be confused with other adnexal lesions, only one false-positive diagnosis of an ectopic sac being made in this series. The presence of a pelvic haematoma on ultrasound, seen in three patients, while not diagnostic of an ectopic pregnancy, is clearly pathological and warrants surgery no matter what the cause. While the subtle pelvic findings of patients suspected of having an ectopic pregnancy may be assessed on transabdominal ultrasound (de Crespigny 1987), definitive diagnosis using this route is less likely.

Demonstration of the ectopic pregnancy with transvaginal ultrasound allows a more precise pre-operative diagnosis, so is likely to allow earlier surgical intervention with resultant minimization of tubal damage. Unnecessary surgery because of an unrecognized recent miscarriage or a 'missed' intrauterine pregnancy on ultrasound should occur less often. Transvaginal ultrasound also allows diagnosis of intrauterine pregnancy earlier than it is normally seen by transabdominal ultrasound, and the improved images mean intrauterine 'pseudosacs' should no longer cause misdiagnosis.

Vaginal ultrasound has been demonstrated to be superior to transabdominal ultrasound in the assessment of patients suspected of having an ectopic pregnancy. While laparoscopy may still

Table 1. Diagnostic efficacy of transvaginal ultrasound in 148 suspected ectopic pregnancies

Ultrasound diagnosis	Outcome			
	Intrauterine pregnancy	Ectopic pregnancy	Not pregnant	Total
Intrauterine pregnancy	72	—	—	72
Ectopic pregnancy	—	29	2	31
Not pregnant	3	7	35	45
Total	75	36	37	148

be required for the patient with a persistently positive pregnancy test with neither a demonstrable intrauterine pregnancy nor a pelvic mass on ultrasound, gynaecologists should be circumspect about resorting to surgery as the ectopic pregnancy or its haematocele is visible in most patients with an ectopic pregnancy.

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Secular trend in the rate of small-for-gestational-age infants: Haguenau Study 1971–1985

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Summary. Between 1971 and 1985, at the Haguenau Maternity Hospital, a 20% decrease was observed in the rate of small-for-gestational-age infants (birthweight <10th centile using figures from all the study births) among 20 101 births between 28 and 42 weeks. This decrease could not be explained by simultaneous changes in socio-demographic and anthropometric characteristics of the pregnant women, nor a recruitment bias. The earliness of prenatal care at the Maternity Hospital may have played a role in the downward trend. Further research is needed to confirm the influence of prenatal care on the rate of small-for-gestational-age babies.

Low birthweight-for-gestational-age depends on several factors, some of which are inherent to the pregnant woman, such as parental stature (Lazar *et al.* 1975), parity (Milner & Richards 1974), or socio-economic status (Ounsted & Scott 1982). Other factors are behavioural or could be changed by medical intervention. Most well known are cigarette smoking (Martin & Bracken 1986), pregnancy weight gain and nutrition (Abrams & Russell 1986) and severe pre-eclampsia (Fedrick & Adelstein 1978). Some pregnancy infections, fetal chromosome abnormalities and severe malformations are also often associated with a low birthweight-for-gestational-age (Chiswick 1985). Increased perinatal mortality risk and long-term impaired function have been described among small-for-gestational-age (SGA) newborns (Butler 1974).

The Perinatal Haguenau Study began in 1971 to assess a preterm prevention programme. The programme consisted of early prenatal care at the Haguenau Maternity Hospital, identification of each woman's risk factors for preterm birth, and reduction of the woman's physical activity (Papiernik 1984). Besides the decrease in the preterm rate already described (Papiernik *et al.* 1985), this research gave us the opportunity to study the time trend in the proportion of SGA newborns. More specifically, the objective was to investigate the influence of changes in obstetric practice and prenatal care on the SGA rate, while attempting to control for simultaneous shifts in socio-demographic and anthropometric characteristics of the pregnant women.

Patients and methods

The Haguenau Maternity Hospital is the main maternity hospital in the Haguenau area. Census data, which were studied from 1971 to 1983

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(Bouyer *et al.* 1987), show that 90% of the births from residents of the city of Haguenau and 80% of births from residents of the surrounding areas took place in the hospital. An analysis of time trend was also performed and showed the stability of the study population, apart from a slight increase in women of low socio-economic status. This slight deviation resulted from the closure of two small local maternity hospitals.

From February 1971 to December 1985, 21 003 singleton births were registered; gestational age was unknown in 590. All of the 20 118 deliveries at between 28 and 42 completed weeks gestation were selected for this study, except 17 where birthweight was unknown. Births before 28 weeks ($n = 34$) or after 42 weeks ($n = 261$) were excluded from this analysis because they were two few for stable birthweight standards to be derived for those gestational ages. Moreover, at these extreme gestations dramatic changes occurred either in obstetric practice (induction of nearly all pregnancies after 42 completed weeks) or in reporting practice (distinction between late abortion or perinatal death). Preterm births were defined as births occurring before 37 completed weeks since the last menstrual period.

Most variables characterizing pregnancy and delivery were registered from 1971. Term was defined by the Maternity Hospital team from the time of last menstruation and, if available, an early ultrasound measurement. Information concerning maternal height, weight and smoking habits at the beginning of the pregnancy was recorded. Socio-economic status was coded as 'low' when maternal schooling comprised less than 8 full years. For this study, the start of antenatal care at the Maternity Hospital was defined as the trimester when the patient first came (first, second, third, or women who first came just for the delivery). Precise data about antenatal care sought in private consulting rooms were not available for the whole 15-year period but it is noteworthy that until 1983 there was no private obstetrician in Haguenau. For the definition to be uniform for all antenatal care, pre-eclampsia was defined as a systolic blood pressure above 140 mmHg or a diastolic above 90 mmHg at the time of delivery.

To account for the specificity of the Haguenau population, local birthweight-for-gestational-age standards according to sex were derived from the livebirths over the 15-year period. The actual 10th centile values were weighted by the number of births at every gestational age. The 10th smoothed centile standard was then used as a frame of reference for measuring the evolution of the SGA rate. A 5th centile was also computed from the births above 30 weeks as there were too few births between 28 and 30 weeks.

The evolution over time of the SGA rate and its risk factors were presented in five 3-year periods and the rates were tested with χ^2 and trend tests. SGA standards were smoothed by using cubic polynomial weighted least squares regression with BMDP statistical software. The time trend of the SGA rate was adjusted on risk factors by stepwise discriminant analysis with SGA as the dependent variable and time, in years, as one of the independent variables (Mardia *et al.* 1982).

Results

The time trend in the SGA rate is illustrated in Table 1. The percentage of infants below the 10th centile has significantly decreased during the 15-year study period. Among them, the proportion of newborn infants below the 5th centile between 31 and 42 weeks has also decreased ($P<0{\cdot}05$). The downward trend in the SGA rate was seen among the 19 181 infants born at term and the 546 born between 35 and 36 weeks, and was particularly marked during the period 1971–1982. On the other hand, the rate of SGA infants born at less than 35 weeks seems to have increased with time, although the difference was not statistically significant. It can be seen from Table 1 that low birthweight (<2500 g) and very low birthweight (<1500 g) rates followed the same pattern. Mean birthweight did not change during these 15 years but the dispersion was reduced.

This time trend could not be explained by a simultaneous decrease in the incidence of severe malformations. Among livebirths without any severe abnormality such as anencephaly, micro- or hydrocephaly, severe neural tube defect or chromosomal abnormality, a slightly less pronounced decrease in the SGA rate was also found from 10·6% in the first period to 9·2% in the last one.

The association between growth retardation and well-known risk factors was verified. Maternal age below 21 years or above 35, parity >3, <8 years maternal schooling, height <1·61 m, weight ⩽55 kg and pregnancy weight gain of <8 kg, were all associated with an increased risk

Table 1. Trend with time of mean birthweight and percentage of small-for-gestational-age (SGA) among 28 to 42 weeks births at the Haguenau Maternity Hospital, 1971–1985

	1971–73	1974–76	1977–79	1980–82	1983–85
Number of births	4185	3521	3811	4483	4118
Birthweight (g) Mean (SD)	3352 (545)	3381 (522)	3367 (515)	3379 (494)	3330 (489)
Preterm rate (%)	5·76	4·86	3·91	4·08	4·69
LBW rate (<2500 g) (%)	4·97	4·32	4·30	3·75	4·40
VLBW rate (<1500 g) (%)	0·84	0·48	0·55	0·27	0·32**
SGA babies *n* (%)					
28–34 weeks	8 (7·1)	8 (10·0)	11 (15·1)	7 (12·3)	8 (12·5)
35–36 weeks	23 (18·1)	13 (14·3)	13 (17·6)	8 (6·4)	14 (10·9)*
37–42 weeks	439 (11·1)	335 (10·0)	367 (10·0)	364 (8·5)	358 (9·1)**
Total	470 (11·2)	356 (10·1)	391 (10·3)	379 (8·5)	380 (9·2)**
Proportion of VSGA among SGA babies	0·55	0·54	0·51	0·48	0·49

* $P<0.05$, ** $P<0.001$ (χ^2).

SGA, Small-for-gestational-age (<10th centile); VSGA, very-small-for-gestational-age (<5th centile) between 31 and 42 weeks, LBW, low-birthweight; VLBW, very-low-birthweight.

of giving birth to an SGA infant. Women who smoked before pregnancy also had an increased risk which was proportional to the daily number of cigarettes smoked. The percentage of SGA babies was 11% in women whose blood pressure was raised at delivery. Women who sought early prenatal care at the Maternity Hospital, in the first trimester, had an 8·5% risk of giving birth to an SGA baby. This risk increased with the time of first consultation to 12% among women who came in just for the delivery. More SGA babies were born after induced (11%) than after spontaneous onset of labour (9·6%).

The time trend in the distribution of these factors is illustrated in Table 2. The socio-demographic and anthropometric characteristics of the pregnant women changed. Over the years, there were fewer patients who were small, aged <21 or >35 years, or of parity ≥4. Pregnant women's behaviour changed too. The percent-

Table 2. Trend in time of the distribution of factors associated with growth retardation—Haguenau Maternity Hospital, 1971–1985

	1971–73		1974–76		1977–79		1980–82		1983–85	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Age (years)										
≤20	683	(16·4)	652	(18·7)	582	(15·4)	594	(13·3)	402	(9·9)*
≥36	371	(8·9)	189	(5 4)	117	(3·1)	130	(2·9)	147	(3·6)*
Parity										
0	1696	(40·5)	1674	(47·5)	1787	(46·9)	1946	(43·4)	1809	(43·9)
≥4	462	(11·0)	219	(6·2)	178	(4·7)	174	(3·9)	159	(3·9)*
Height ≤1·60 m	1617	(46·3)	1516	(44·4)	1683	(45·7)	1907	(42·7)	1736	(42·5)*
Weight ≤55 kg	1692	(45·0)	1569	(46·3)	1772	(48·5)	2022	(46·1)	1859	(45·5)
Low schooling	2711	(64·8)	2284	(64·9)	2528	(66·3)	3228	(72·0)	2599	(63·1)
Smokers	387	(9·3)	490	(13·9)	671	(17·6)	737	(16·4)	913	(22·2)*
High blood pressure	124	(10·3)	380	(11·4)	349	(9·5)	585	(14·0)	319	(8·7)
Weight gain ≤7 kg†	576	(19·9)	777	(25·0)	746	(21·8)	777	(19·3)	683	(18·9)*
Late prenatal care	2135	(51·0)	1642	(46·6)	1361	(35·7)	916	(20·4)	1232	(32·0)*

* *P* trend $<10^{-5}$.

† In term deliveries where weight gain known.

age of women with a low pregnancy weight gain fell, even allowing for the decrease in the preterm rate during this period. Late prenatal care at the Maternity Hospital (women who first attended during the third trimester or for delivery) fell until 1982. On the other hand, the proportion of women who smoked before pregnancy increased with time, as did the average number of cigarettes smoked, from 6·7 a day at the beginning of the study to 10·0 at the end. Elective deliveries (following induction or by caesarean section) increased at all gestational ages from 4·2% in 1971–3 to 22·7% in 1983–5.

When stratified by all risk factors, the decline in the SGA rate was relatively uniform in all classes, although the differences were not always statistically significant. For women of parity ≥4 no trend at all was observed.

To account for time changes in the frequencies of the risk factors described in Table 2, the decline in the SGA rate in births at between 35 and 42 weeks was adjusted by a stepwise forward multivariate analysis. Table 3 indicates factors that significantly discriminated the dependent variable, SGA. The first model failed to explain the decline in the SGA rate by anthropometric and socio-demographic changes in pregnant women: maternal height and weight, parity and schooling could not rule out the influence of time as a discriminant factor. When the date of first consultation to the Maternity Hospital was introduced in a second model, the time no longer played a discriminant role. In order to adjust the start of prenatal care on possible confounding variables, maternal weight, smoking habits, pregnancy weight gain, nulliparity, height and blood pressure at delivery were introduced in the model. The earliness of prenatal care was still a significant factor in the explanation of the decline of the SGA rate. Maternal schooling was still discriminant, but maternal age no longer played any significant role.

Table 3. Adjustment on risk factors of the trend with time of the SGA rate at the Haguenau Maternity Hospital between 1971 and 1985

Model	Variables entered by forward procedure	Significance P
First	Weight*	$<10^{-6}$
	Height*	$<10^{-6}$
	Nulliparity†	$<10^{-6}$
	Schooling†	$<10^{-5}$
	Year*	0·004
Second	Weight*	$<10^{-6}$
	Height*	$<10^{-6}$
	Nulliparity†	$<10^{-6}$
	Date of first consultation‡	$<10^{-4}$
	Schooling†	$<10^{-3}$
	Year*	0·17
Third	Weight*	$<10^{-6}$
	Smokers†	$<10^{-6}$
	Weight gain*	$<10^{-6}$
	Nulliparity†	$<10^{-6}$
	Height*	$<10^{-6}$
	High blood pressure†	$<10^{-6}$
	Date of first consultation‡	0·007
	Schooling†	0·02
	Year*	0·18

* Continuous variable.
† Coded 0 or 1.
‡ In four classes.

Discussion

This study showed a 20% decrease at the Haguenau Maternity Hospital in the rate of SGA babies between 1971 and 1985. Among these SGA babies, defined as below the 10th centile, the proportion of very-small-for-gestational-age (<5th centile) also decreased. This time trend could not be explained either by simultaneous changes in socio-demographic or anthropometric characteristics of the pregnant women (height and weight, schooling, age, parity). The decrease in the proportion of SGA babies was not related to an overall shift in the distribution of birthweight.

We think the decline observed at the Haguenau Maternity Hospital is actual and not biased by any change in recruitment such as women with an increased risk of giving birth to an SGA baby now being delivered elsewhere. In France, there is no official policy of transfer *in utero* to highly specialized centres. The recruitment is very likely to be community based, with no other hospital in the catchment area. Its representativeness was verified by census records (Bouyer *et al.* 1987). We do not think that the closure of the two small maternity hospitals would have increased in our study population the number of women at low obstetric risk, as the same trend in the SGA rate was observed among residents at the city of Haguenau delivered at the Maternity Hospital; these women consistently represent 92% of all the pregnant women in the city.

It is unlikely, too, that this decline in the SGA rate could be attributed to any systematic change in the assessment of gestational age (Bouyer *et al.* 1987). This study was started in 1971 to assess a preterm prevention programme. Gestational age was carefully estimated and registered. All preterm files were reviewed by the obstetric team. The decrease observed in the preterm rate was accompanied by an associated decrease in the proportion of newborn infants weighing <2500 g (Papiernik *et al.* 1985). The proportion of cases for which gestational age was unknown decreased with time, but their mean birthweight did not change significantly. It is very unlikely that the exclusion of these 590 births could explain the observed trend in the SGA rate.

The birthweight standard we used to define SGA was built specifically from the characteristics of the newborn infants at the Maternity Hospital, to reflect the pattern of fetal growth in that population, as recommended by other authors (Carr-Hill & Pritchard 1983; Forbes & Smalls 1983). This empirical internal standard was used solely to assess the trends in Haguenau and should not be applied to other populations without caution.

The decline in the SGA rate between 1971 and 1985 at the Haguenau Maternity Hospital can be compared with the results of the French Perinatal Study (Rumeau-Rouquette *et al.* 1984). They observed a decrease in the proportion of SGA infants (defined by the 5th centile) born at term from 5·1% in 1972 to 3·6% in 1981. International comparisons are difficult to make, because of the lack of uniformity in definitions, reporting practices, or study period. In the USA, Kessel *et al.* (1984) described a 20% decrease in the rate of SGA babies (defined as term births ≤2500 g) between 1970 and 1980 among white women. They did not have enough detailed information to assess whether this trend was associated with any trends in anthropometric or socio-demographic variables. Alberman (1985) in England and Wales reported no variation in the low-birthweight rate between 1979 and 1983, which was very similar to the rates described by Peters *et al.* (1983) in 1958 and in 1970. Unfortunately, these latter studies gave no data concerning birthweight related to gestational age.

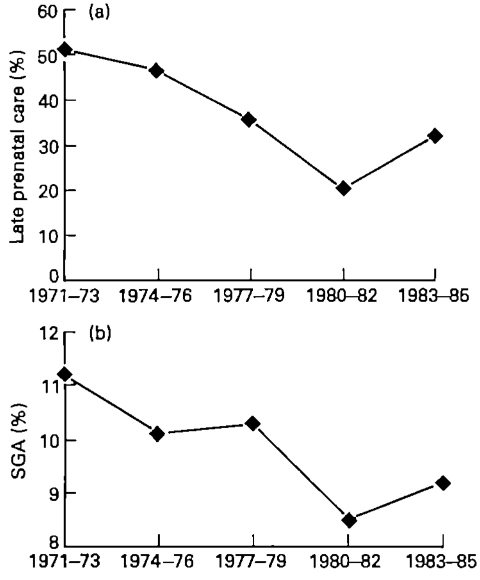

Fig. 1. Time trends for (a) prenatal care and (b) SGA rates at Haguenau Maternity Hospital.

The present study allowed us to generate some hypotheses concerning the influence of some elements on the SGA rate. The earliness of prenatal care at the Maternity Hospital appeared to play a role in the downward trend of the SGA rate. With the passage of time, there were fewer women who attended the hospital only in the late stages of pregnancy. Their distribution over time was quite similar to that of the SGA rate, that is a downward trend until 1982 and a slight increase afterwards (Fig 1). In an individual analysis, the risk of giving birth to an SGA baby increased with the trimester of first consultation at the Maternity Hospital. This relation persisted when adjusted for confounding variables such as maternal age, parity, schooling and height. A recruitment bias could have occurred, in that some women at increased risk of giving birth to an SGA infant could have sought very early prenatal care at the Maternity Hospital. This bias, if it occurred, would have caused this association between SGA and prenatal care to be underestimated.

Associations between prenatal care and the preterm rate (Papiernik *et al.* 1986), birthweight adjusted for length of gestation (Showstack *et al.* 1984) or risk of a low birthweight baby (Gortmaker 1979) have been described. The question is how to discover which specific aspects of prenatal care may have led to this decline in the SGA rate, as few determinants of fetal growth are known (Cnattingius *et al.* 1984). A number of severely growth-retarded babies have probably been avoided by improved antenatal screening and therapeutic abortion. This was mentioned by Kleinman *et al.* (1978) to explain a part of the decrease in early neonatal mortality in the USA between 1960 and 1973–4, and confirmed by Alberman (1985) between 1975 and 1979 in England and Wales. In our study, we observed an increase with time in the number of therapeutic abortions, a decrease in the rate of very-small-for-gestational-age infants, and a decrease in the percentage of babies with severe malformations or death *in utero*. Nevertheless, this could not explain all of the decrease in the SGA rate as it was still observed among livebirths without severe malformations. The influence of the earliness of prenatal care in the multivariate analysis was not explained by accounting for both pregnancy weight gain (which has increased with time) and blood pressure at the time of delivery. Pre-eclampsia was not as satisfactorily defined in this study (blood pressure at the time of delivery) as we would have wished. Nevertheless, as far as we know, there are few claims that changes in the treatment of pre-eclampsia during recent years could have improved birthweight. Although the reliability of screening for fetal growth retardation is still a matter of controversy (Benson *et al.* 1986), induction has been suggested in many studies as an explanation for the change of birthweight distribution (Keirse 1984). Induction would decrease the proportion of SGA babies born at term and increase the proportion of SGA infants in induced preterm births. In our study, the percentage of induced births was greater in women who came to the Maternity Hospital before delivery. The reasons for induction had not been registered in all years, so we could not verify this important hypothesis. There is an indirect argument against it: the downward trend in the SGA rate was still significant when, on a conservative hypothesis, we considered that the 210 induced preterm births would have been born SGA at term had delivery not been induced. An alternative explanation for the downward trend with time of the SGA rate can be advanced when remembering that the preterm prevention programme included reduction of the patients' physical activity and eventually stopping work. These measures, initially directed towards the prevention of preterm births, could have had an impact on fetal growth.

The downward trend in the percentage of SGA babies was observed essentially in term births and, slightly less, in births at 35–36 weeks. Among babies born at <35 weeks gestation, an increase seemed to have occurred with time although this was not statistically significant. With improvements in neonatal care, obstetricians may have allowed some babies to be born at an earlier gestational age where severe fetal distress was suspected. Concurrently, the decrease in the preterm rate observed over the same period may have concerned those pregnancies where fetal growth was not affected, involving a selection process (Carr-Hill & Pritchard 1985). It must be kept in mind that our definition of growth retardation should be used with caution in very preterm births that are, by definition, abnormal in some way (Alberman 1984). The neonatal mortality rates were respectively 0·74%, 0·48%, 0·45%, 0·20% and 0·25% in the five periods of this study. Of course it would have been very interesting to be able to relate the observed decrease in the SGA rate to a decrease in neonatal mortality or morbidity. It was not attempted because it was very difficult in this study of time trends to separate the effects of changes in prenatal care from those of improvements in perinatal monitoring and neonatal care. Conversely, gestation at delivery and birthweight could be influenced, if at all, only by prenatal care.

Between 1983 and 1985, a slight increase in the SGA rate seems to have occurred. Only further observation will enable us to decide whether this was a random fluctuation or an actual increase. If the evolving patterns of the SGA rate and the late presentation for prenatal care at the Maternity Hospital remain parallel, this will support our inferences about the influence of early prenatal care at the Maternity Hospital on the risk of giving birth to an SGA baby.

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Antenatal serum levels of relaxin in patients having preterm labour

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Summary. This study is the first report of antenatal levels of relaxin measured by homologous radioimmunoassay in peripheral serum from patients who subsequently had a preterm delivery. Serial blood samples were collected antenatally from a group of subjects known to be at increased risk of preterm labour because of a past history of shortened pregnancy. Serum relaxin was measured using an homologous radioimmunoassay based on a synthetic bioactive analogoue of the native hormone. In women whose pregnancies ended preterm most measurements were within the range of values previously established in normal antenatal patients although some measurements early in pregnancy were above the normal range. These findings suggest that low serum levels of relaxin are not causatively related to the onset of labour before term.

It has been reported that the application of an extract of the human corpus luteum of pregnancy to human myometrium caused a decrease in the amplitude of spontaneous contractions *in vitro*. The extract was shown to contain immunoreactive and bioactive relaxin (Szlachter *et al.* 1980). However, the clinical significance of the role played by human relaxin in maintaining quiescence of the pregnant myometrium *in vivo* is unknown. It has been reported that the mean serum level of immunoreactive relaxin (measured using a heterologous assay for porcine relaxin) in a group of subjects admitted in preterm labour was significantly lower than the mean level in gestation-matched controls who were not in labour (Szlachter *et al.* 1982). Relaxin levels were not available from the study group during the antenatal period.

The aim of this investigation was to document the levels of relaxin in peripheral serum throughout pregnancy in subjects who subsequently were delivered prematurely, using a radioimmunoassay based on an analogue of human relaxin. Antenatal, rather than intrapartum levels should determine whether preterm labour is preceded by abnormal relaxin levels, which could be related causally to the onset of labour before term, and whether the measurement of relaxin levels during pregnancy would be useful in determining the gestation at which labour will occur.

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Patients and methods

The patients described in this study were attending clinics at the Queen Victoria Medical Centre. The study was approved by the human ethics committees of the Howard Florey Institute and the Queen Victoria Medical Centre. All patients participating did so voluntarily after having given written consent.

Gestational age was measured in completed weeks from the first day of the last normal menstrual period (LMP) and was confirmed either by the findings of a clinical examination in the first trimester being consistent with the period of amenorrhoea or an early ultrasound examination. For women delivered before term, gestation at delivery was confirmed by the neonatal assessment of maturity of the infant. Term deliveries included births between 37 and 42 completed weeks. Preterm deliveries included all liveborn and stillborn infants born between 20 and 37 completed weeks. Spontaneous delivery before 20 weeks gestation was termed miscarriage.

Patients were selected for this study on the basis of at least one previous shortened pregnancy, either preterm delivery or mid-trimester miscarriage. This past obstetric history is the single most important risk factor for the identification of patients who will subsequently be delivered before term (Keirse *et al.* 1978; Bakketeig *et al.* 1979). Of the 25 subjects originally recruited, 22 were followed to delivery. Three patients withdrew from the study.

Of the 22 patients who completed the study, five were delivered preterm at between 21 and 35 weeks and six had a miscarriage at between 10 and 19 weeks. Ten patients were delivered at term and one at 43 weeks.

Serial blood samples were collected every 4 weeks from the time each patient presented to the antenatal clinic until 34 weeks, and then every 2 weeks until delivery.

Blood was collected directly into evacuated plain glass tubes. It was centrifuged after clotting at room temperature and the serum stored in triplicate at −20°C until assayed.

The details of the assay have been published previously (Eddie *et al.* 1986). Serum relaxin was measured in duplicate by radioimmunoassay (RIA). Rabbit antiserum to a synthetic bioactive analogue of human relaxin was used at an initial dilution of 1 in 6000. The same relaxin analogue was used as standard and radioiodinated for use as a tracer. No cross-reactivity was detected with bovine insulin, human chorionic gonadotrophin, chorionic somatomammotrophin or growth hormone. Sensitivity of the assay was 0·06 ng/ml. The intra-assay coefficient of variation was 3·83% ($n = 22$) at concentrations of 1·57, 0·76 and 0·69 ng/ml. The interassay coefficient of variation for samples at 2·06 ng/ml was 9·6% ($n = 10$).

Results

Five high-risk patients were delivered preterm. The relaxin levels in these patients relative to the reference range previously established for normal patients (Bell *et al.* 1987) are shown in Fig. 1. Levels >2 SD above the reference mean were found in four of the five subjects (four out of 18 samples). All other results were within the reference range (mean ± 2 SD). One subject (B24) had blood collected during preterm labour at 35 weeks gestation. The relaxin level in this patient during labour (0·68 ng/ml) was very similar to the level measured 3 days earlier (0·69 ng/ml). The time between the collection of the last blood sample and the onset of labour in the other four subjects ranged from 3 to 21 days.

Six pregnancies ended in miscarriage. In five of the six subjects only one sample was collected, because of the short time interval between recruitment of subjects and miscarriage. The relaxin level in one subject (B23) was >2 SD above the reference mean. All other measurements in this group lay within the reference range.

Discussion

This is the first report of serum relaxin levels in pregnancies delivered preterm in which relaxin immunoactivity was determined by a radioimmunoassay based on an analogue of human relaxin. It is also the first time relaxin immunoactivity has been measured during the antenatal period in subjects who subsequently were delivered before term.

It has been reported by a group using a heterologous assay that the mean level of relaxin immunoactivity in serum from subjects admitted between 29 and 36 weeks gestation in preterm labour was significantly lower than the mean level in women in the third trimester who were not in labour (Szlachter *et al.* 1982). But the difference was not significant when only those

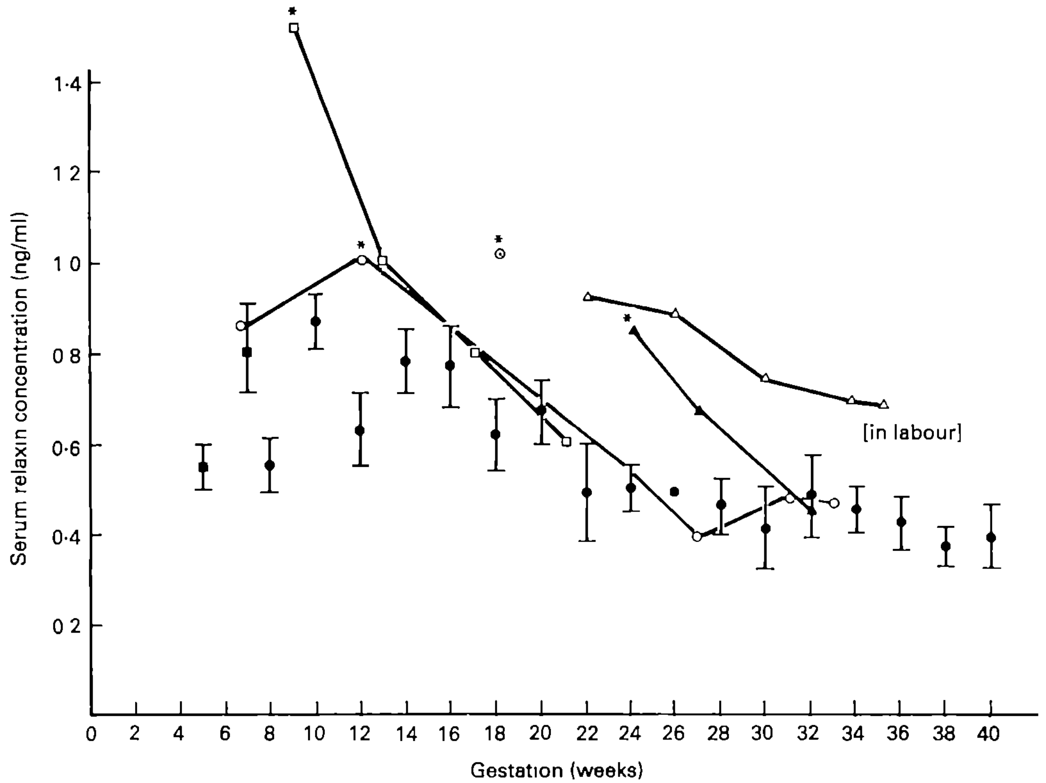

Fig. 1. Serum relaxin levels in five subjects subsequently delivered preterm [subject B5 (○——○) at 33 weeks; B10 (▲——▲) at 35 weeks; B13 (⊙) at 21 weeks; B21 (□——□) at 22 weeks; and subject B24 (△——△) at 35 weeks shown in relation to a reference range (mean ± SEM) determined in a normal population (Bell *et al.* 1987) (♦, ▮). Four subjects had relaxin values >2 SD above the reference mean (shown by *) at 10, 12, 18 and 24 weeks (the numbers of measurements used to determine the reference range at these gestations were 11, 5, 9 and 10 respectively)

patients who were delivered within 24 h of admission (thus excluding patients in spurious labour) were compared with controls. Despite the difference between the mean values of the groups almost all of the individual serum levels in the preterm group would lie within the reference range of control subjects. These observations are not consistent with a low serum level of relaxin being related causally to preterm labour.

The results of the current study suggest that antenatal levels of relaxin in patients who subsequently have a shortened pregnancy are not low. Indeed, some measurements were above the reference range for control subjects. The physiological significance of these high levels is not known. It has been suggested that a low level of relaxin could decrease uterine quiescence and so cause premature labour (Szlachter *et al.* 1982). It has also been suggested that high levels of relaxin could cause preterm labour by a relaxin-induced form of cervical incompetence (Haning *et al.* 1985).

Antenatal data on patients who subsequently give birth preterm are difficult to obtain because these subjects are extremely difficult to identify within the antenatal population. The results of this study suggest that low serum levels of relaxin during the antenatal period are not causatively related to the onset of preterm labour.

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Placental histology and the production of human choriogonadotrophin and its subunits in pregnancy

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Summary. The relation between placental histology and the levels of human chorionic gonadotrophin (hCG) and its α-hCG and β-hCG subunits was studied using monoclonal antibodies in immunohistochemical and immunoassay procedures. Between 4 and 9 weeks gestation an increasing ratio of cytotrophoblasts to syncytiotrophoblasts in placental villi corresponded to rising hCG levels, with βhCG levels declining from 4% to less than 1% of hCG levels. After 8–10 weeks gestation, declining numbers of cytotrophoblasts in villi and the formation of the definitive placenta coincided with falling hCG, rising αhCG and insignificant βhCG levels. Between 18 and 35 weeks, a secondary twofold rise in hCG levels coincided with a similar increase in average placental mass. It is proposed that hCG synthesis in syncytiotrophoblasts depends on the rate of differentiation of cytotrophoblasts into syncytiotrophoblasts.

Choriogonadotrophin (human chorionic gonadotrophin, hCG) is the predominant placental hormone secreted in early pregnancy, with maximum hCG levels in the first trimester (Loraine & Matthew 1953; Braunstein *et al.* 1976). This is in contrast to the secretion of many other placental hormones, such as human placental lactogen (hPL) and pregnancy-specific β-1-glycoprotein, whose levels in maternal sera increase progressively during pregnancy (Braunstein *et al.* 1980). Consequently, hCG levels cannot be correlated with the growth of trophoblastic mass and do not increase at a fixed rate per unit of placental mass. The mechanisms that control the synthesis and secretion of hCG are unclear at present.

The recent development of monoclonal antibodies has introduced greater specificity and sensitivity in the measurement of hCG and its alpha (αhCG) and beta (βhCG) subunits. Previous studies of hCG profiles with polyvalent antisera have reported conflicting findings regarding both a secondary hCG rise in the last trimester (Braunstein *et al.* 1976; Ashitaka *et al.* 1980) and the presence of βhCG in maternal serum. Groups have reported finding insignificant amounts of βhCG (Franchimont *et al.* 1972), rising βhCG at the time of peak hCG levels (Harrison *et al.* 1980) or significant levels during the earliest stages of pregnancy (Cole *et al.* 1984).

In the present study, the profiles of hCG and its subunits during normal pregnancy were determined with monoclonal immunoassays. Specific hCG monoclonal antibodies were also used in an immunohistochemical study of placental tissue collected from 3 to 38 weeks gestation. Correlating these two lines of investigation showed that the dynamics of hCG production in pregnancy reflect changes occurring in placental histology and morphology. From these findings a hypothesis is proposed that hCG synthesis is regulated through the rate of differentiation of cytotrophoblasts into syncytiotrophoblasts. A possible mechanism for this control is through variations in concentration of presynthesized messenger ribonucleic acid (mRNA) introduced

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into the syncytium by differentiating cytotrophoblasts.

Materials and methods

Overall, 500 samples of maternal sera were collected serially at approximately monthly intervals from 55 clinically normal women with singleton pregnancies, whose gestational ages (3 to 40 weeks) were assessed from the last menstrual period (LMP), and by ultrasonography. Serum aliquots were stored at −70 °C before being assayed in randomized batches. Trophoblastic material was collected after curettage at between 3 and 16 weeks gestation, and at between 16 and 40 weeks gestation after vaginal delivery or caesarean section. This material was fixed in 10% buffered formalin, routinely processed and embedded in paraffin.

Immunoassays used to measure αhCG, βhCG and dimer hCG levels have been described previously by Hay (1985). Highly specific monoclonal antibodies for αhCG (clone no. 63/5) and βhCG (clone no. 1/9) were used in an immunoradiometric assay (IRMA) supplied by Bioclone Australia, Sydney, Australia. Dimer hCG was measured in a modified commercial IRMA marketed by Hybritech, Inc., San Diego, CA, USA. Assay standards were the first International Reference Preparation for dimer hCG (IRP 75/537), αhCG (IRP 75/569) and βhCG (IRP 75/551), supplied by the National Biological Standards Board, London, UK. All immunoassays had a minimum sensitivity of 0·5 μg/l. The intra-assay coefficients of variation (CV) at 30 μg/l for αhCG, βhCG and dimer hCG were 10·5%, 9·5% and 8·7%; and the intra-assay CVs were 8·5%, 7·9% and 4·5%, respectively. The binding characteristics of the monoclonal antibodies used in this study have been reported previously (Stuart *et al.* 1983). Briefly, in the βhCG assay, dimer hCG and αhCG cross-reacted at 0·3% and 0·7%, respectively; whereas in the αhCG assay, dimer hCG and βhCG cross-reacted 0·06% and 0·1%, respectively. In the IRMA assay for dimer hCG, no significant cross-reactions were found for either αhCG or βhCG.

The sites of hCG and αhCG synthesis in placental tissue were localized by a four-layer bridge immunoperoxidase technique described in detail by Wahlstrom *et al.* (1981). Tissue sections cut from paraffin blocks were deparaffinized and cleared through xylene and graded washes to distilled water. Following quenching of endogenous peroxidase activity, the slides were incubated with a primary monoclonal antibody to hCG (clone 26/1) or to αhCG (clone 63/5). The sections were then sequentially incubated with rabbit anti-mouse immunoglobulin, swine anti-rabbit immunoglobulin and rabbit peroxidase-anti-peroxidase. The colour reaction was developed with the peroxidase chromogen 3-amino-9-ethylcarbazole and hydrogen peroxide.

To examine the cytotrophoblast–syncytiotrophoblast distribution during pregnancy, estimates were made of the average width of the syncytium and the cytotrophoblast layers in trophoblastic villi at different gestational ages. These measurements were made from photographic prints of immunostained placental tissue after correcting for magnification in microscopic lens and in the printing negatives. The approximate widths were calculated in selected villi in which the section was perpendicular to the plane of the syncytial surface. The relation between hCG levels and trophoblast mass at different gestations was studied using previously published data on placental weights (Hamilton & Hamilton 1977; Braunstein *et al.* 1980). It was not possible to perform morphometric studies on placentas derived from the same patients who contributed serum samples.

Levels of hCG and subunits for a particular gestation did not show a Gaussian distribution and the normal ranges were summarized by the 5th, 50th (median) and 95th centiles. The median levels obtained at different gestations were compared, and the significances in their differences calculated according to Student's *t*-test for non-paired data. The probabilities were taken from two-tail tables, and values of $P<0 \cdot 05$ were considered significant.

Results

Figure 1 shows the levels of hCG measured in maternal serum from 3 to 40 weeks gestation. There was considerable individual variation both in the rates of increase and peak concentrations. At 4 weeks gestation, mean doubling times of hCG levels were 2·2 (SD 0·8) days compared with 3·5 (SD 1·2) days at 9 weeks. The peak median level of 108 800 i.u./l occurred at 10 weeks. Between 12 and 16 weeks, median hCG levels decreased rapidly with a mean halving time of 2·5 (SD 1·1) days to become 25% of peak

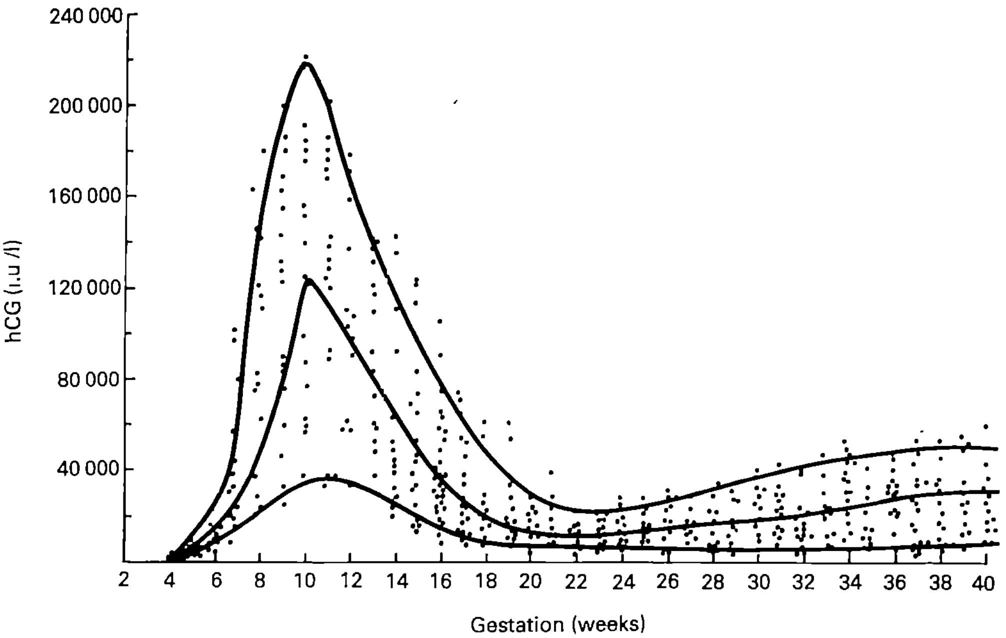

Fig. 1. Dimer hCG levels (i u./l) in 500 serum samples collected between 3 and 40 weeks gestation (LMP) from 55 patients showing 5th, 50th and 95th centile.

values. Levels continued to fall, from 16 to 22 weeks, at a slower mean halving rate of 4·1 (SD 1·8) days to become 10·7% of peak median hCG levels. During the third trimester there was a statistically significant rise in median hCG levels. When median hCG values were grouped together between 22–25, 26–30, 31–34 and 35–38 weeks, the median hCG levels of these groups were 14 960, 19 648, 20 804 and 22 952 i.u./l, respectively, and represented 13·8%, 18·2%, 19·1% and 21·1%, respectively, of the peak median value. A small secondary peak in hCG production was evident as a significant difference in the median hCG values between the first and last of these four groups ($P<0\cdot05$).

The levels of αhCG and βhCG measured directly in maternal serum are shown in Fig. 2 but cross-reactivity of dimer hCG in the free subunit assays contributed significantly to observed levels. Expressing free hCG subunit levels as a percentage of dimer hCG revealed only small amounts of free subunits were present during pregnancy, as shown in Fig. 3. Thus, βhCG levels measured directly appeared to parallel dimer hCG levels during pregnancy but correcting for dimer hCG cross-reactivity showed the mean ratio of βhCG/dimer hCG decreased from 4 (SD 2)% at 3 weeks to less than 1 (SD 0·4)% at 10 weeks, and remained at less than 1 (SD 0·3)% during the second and third trimester. The median levels of αhCG, however, rose in a sigmoid curve to reach peak levels at 36 weeks. The mean ratio of αhCG/dimer hCG was less than 0·1% between 6 and 12 weeks gestation, increased to 0·9 (SD 0·1)% by 20 weeks, and was maintained at a relatively constant ratio of 1·1 (SD 0·2)% for the remainder of pregnancy. Levels of αhCG remained low relative to dimer hCG levels, being approximately 2000-fold and 150-fold less at 6 and 35 weeks, respectively. Thus, the secondary hCG rise could not be attributed to cross-reaction with αhCG.

The localization of hCG and αhCG in trophoblastic villi is shown in Fig. 4 where hCG is present in the outer syncytial layer and in intermediate cells, whereas αhCG is present in cytotrophoblasts. There is no significant αhCG in the syncytial layer. The changing histology of placental villi during different stages of gestation is shown in Fig. 5. Between 3 and 9 weeks gestation, continuously rising maternal hCG levels are coincident with proliferation of immature trophoblastic villi. The histology of villi during this stage shows an extensive syncytial layer. Between 10 and 18 weeks gestation, declining hCG levels are associated with dramatic reorganization of placental structure to generate

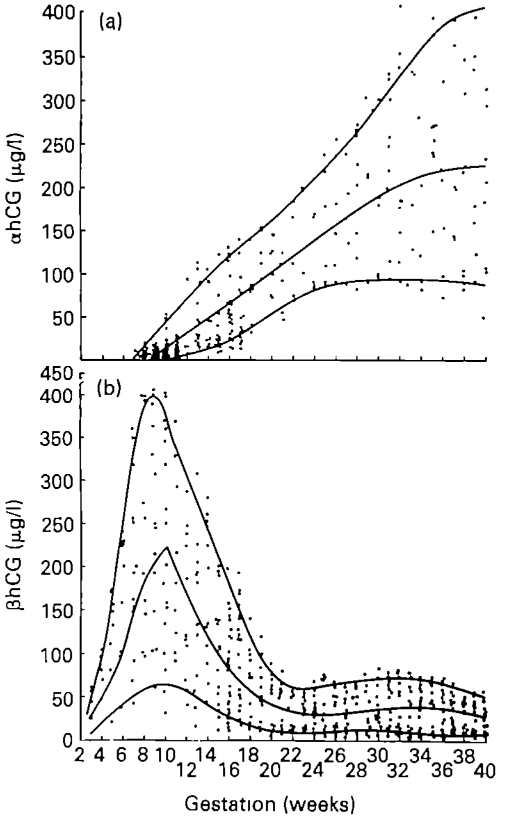

Fig. 2. Serum αhCG (a) and βhCG (b) levels measured directly without correcting for hCG cross-reaction, with 5th, 50th and 95th centiles.

the definitive placenta. During this stage the villi on the decidua capsularis become reduced and placental villi proliferate. The histology of intermediate trophoblastic villi during this stage consists of reduced syncytial layer and declining numbers of cytotrophoblastic cells. From 20 weeks onwards, no further change occurs in the size of the placenta, but there is a gradual increase in its villous volume due to a proliferation of the terminal or fringe villi. In mature villi, which predominate after 25 weeks gestation, the syncytiotrophoblasts are irregularly dispersed and aggregated into 'syncytial knots'. A small percentage (5%) of villi, however, can be classified as immature and represent the growth zone of the villous tree. The small gradual rise in hCG levels in the third trimester may result from increasing villi proliferation within the confines of the definitive placenta.

The approximate width of syncytium and cytotrophoblast layers measured at different gestations is shown in Table 1 Within individual villi the layers of syncytium showed marked variation in width, but a general trend was evident between villi collected at different gestations. Between 4 and 8 weeks gestation the width of the syncytium diminishes progressively by two- to threefold, while the ratio of syncytium width to that of syncytium plus cytotrophoblast widths increased from 4 weeks to peak at 8 to 10 weeks, then declined progressively until 18 weeks. After 20 weeks the syncytium becomes aggregated into knots, making it difficult to calculate the relative thickness of the layers. The changing ratio of syncytium to cytotrophoblasts appears to parallel hCG levels during pregnancy, while falling βhCG levels in early pregnancy correspond to a similar reduction in syncytial width. Also, comparing median hCG levels to published data on placental masses (Hamilton & Hamilton 1977; Braunstein *et al.* 1980) shows the secondary rise in hCG corresponds to a similar increase in placental mass in the third trimester. For example, between 20 and 36 weeks, the median hCG level increased from 15 000 i.u./l to 25 900 i.u./l while median placental mass increased from 110 g to 320 g as detailed in Table 2.

Discussion

The present immunohistochemical study shows a correlation between the proliferating syncytium and hCG levels, and demonstrates a changing distribution of cytotrophoblasts and syncytium during pregnancy which may cause imbalanced secretion of free subunits. As shown in this study, αhCG synthesis is localized to cytotrophoblasts, and hCG synthesis to intermediate and syncytium cells. Thus, the differen-

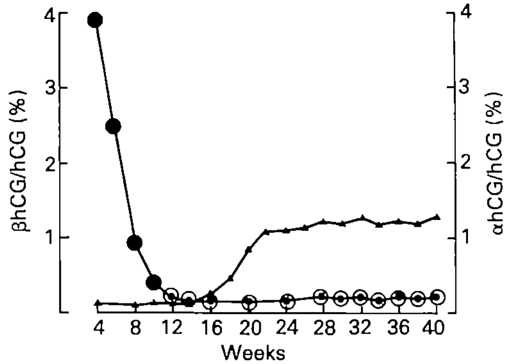

Fig. 3. The mean percentage αhCG/dimer (▲) and βhCG/dimer (●) hCG ratios between 3 and 40 weeks gestation

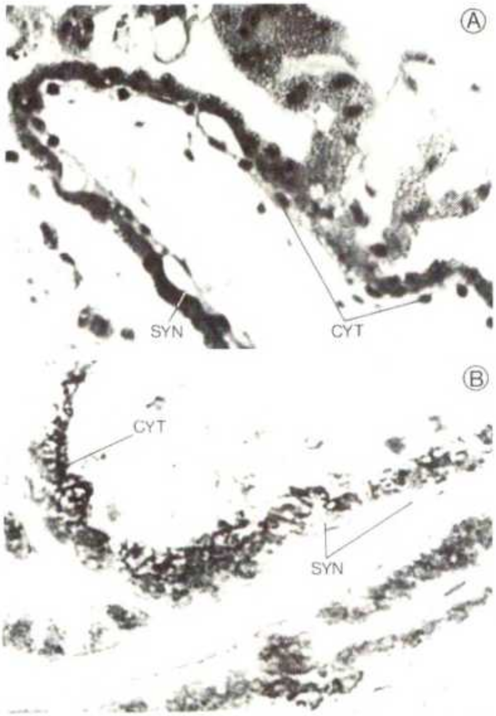

Fig. 4. Localization of hCG synthesis to syncytiotrophoblasts (SYN) in A; and αhCG production only to the inner layer of cytotrophoblasts (CYT) in B.

tiation of cytotrophoblasts into syncytium (Fox 1978; Ilgren 1983) can be linked to the capacity of syncytiotrophoblast to synthesize both βhCG and αhCG, enabling the assembly and secretion of dimer hCG.

The role of trophoblast differentiation in controlling hCG and hPL genes has also been studied using in-situ hybridization with complementary DNA probes (Hoshina *et al.* 1985), confirming previous immunohistochemical findings (Thiede & Choate 1963; Ashitaka *et al.* 1980). Hoshina *et al.* (1985) showed αhCG mRNA was localized to some differentiating cytotrophoblasts and predominantly to syncytial regions, while βhCG mRNA was localized primarily to syncytiotrophoblast regions. Based on these findings, Hoshina *et al.* (1985) proposed that formation of cytotrophoblasts was associated with activation of αhCG gene, while their subsequent differentiation into intermediate cells induced expression of βhCG genes. Furthermore, they proposed that whereas mRNAs for αhCG and βhCG were present in syncytial regions, this did not indicate that hCG was expressed in the mature syncytium. Instead, mRNA for αhCG and βhCG may be localized to multinucleated intermediate cells that could not be resolved from syncytium by present methods. Consequently, cytotrophoblasts regulated hCG production by maintaining a pool of transient intermediate cells whose numbers peaked at 8–10 weeks gestation and declined thereafter.

Assuming that the pathway of cytotrophoblast differentiation regulates hCG mRNA levels, it is possible to propose a different mechanism of control from that of Hoshina *et al.* (1985). For example, it is not necessary to propose that hCG production depends on transcription of hCG genes in intermediate cells in the syncytium. Instead, if hCG genes were not to be transcribed at all in the syncytium, then hCG production would depend on long-acting mRNA introduced by fusion of differentiating cytotrophoblasts. Also, Hoshina's proposal does not consider possible genetic amplification for mRNA-βhCG. Multiple genes exist for βhCG compared to a single gene for αhCG (Fiddes & Talmadge 1984), although not all genes for βhCG are transcribed (Talmadge *et al.* 1984). Genetic amplification, however, would enable rapid accumulation of mRNA for βhCG during the relatively brief stage of differentiation from cytotrophoblasts to syncytiotrophoblasts. Consequently, the concentrations of mRNA for hCG subunits in syncytium could be regulated by altering the rate and number of differentiating cytotrophoblasts.

Using these modifications enables the dynamics of hCG and its subunits in pregnancy to be explained in terms of histological changes in the placenta. For example, in early pregnancy proliferation of primitive trophoblast from rapidly differentiating cytotrophoblasts would result in groups of cytotrophoblasts with insufficient time to accumulate αhCG mRNA equal to βhCG mRNA generated by genetic amplification. If the rate of subunit synthesis in syncytium reflected mRNA concentrations, all αhCG synthesized would be incorporated into dimer hCG and excess βhCG glycosylated and secreted alongside dimer hCG. By 8–10 weeks gestation, extensive villi formation would enable cytotrophoblasts in villi to have optimal rates of differentiation and generate ample amounts of mRNA for both subunits. Thus, the progressive placental development from the primitive syncytial mass to villi formation would be linked to increasing dimer hCG but falling βhCG levels, as shown in the present study. During the second trimester, declining numbers of

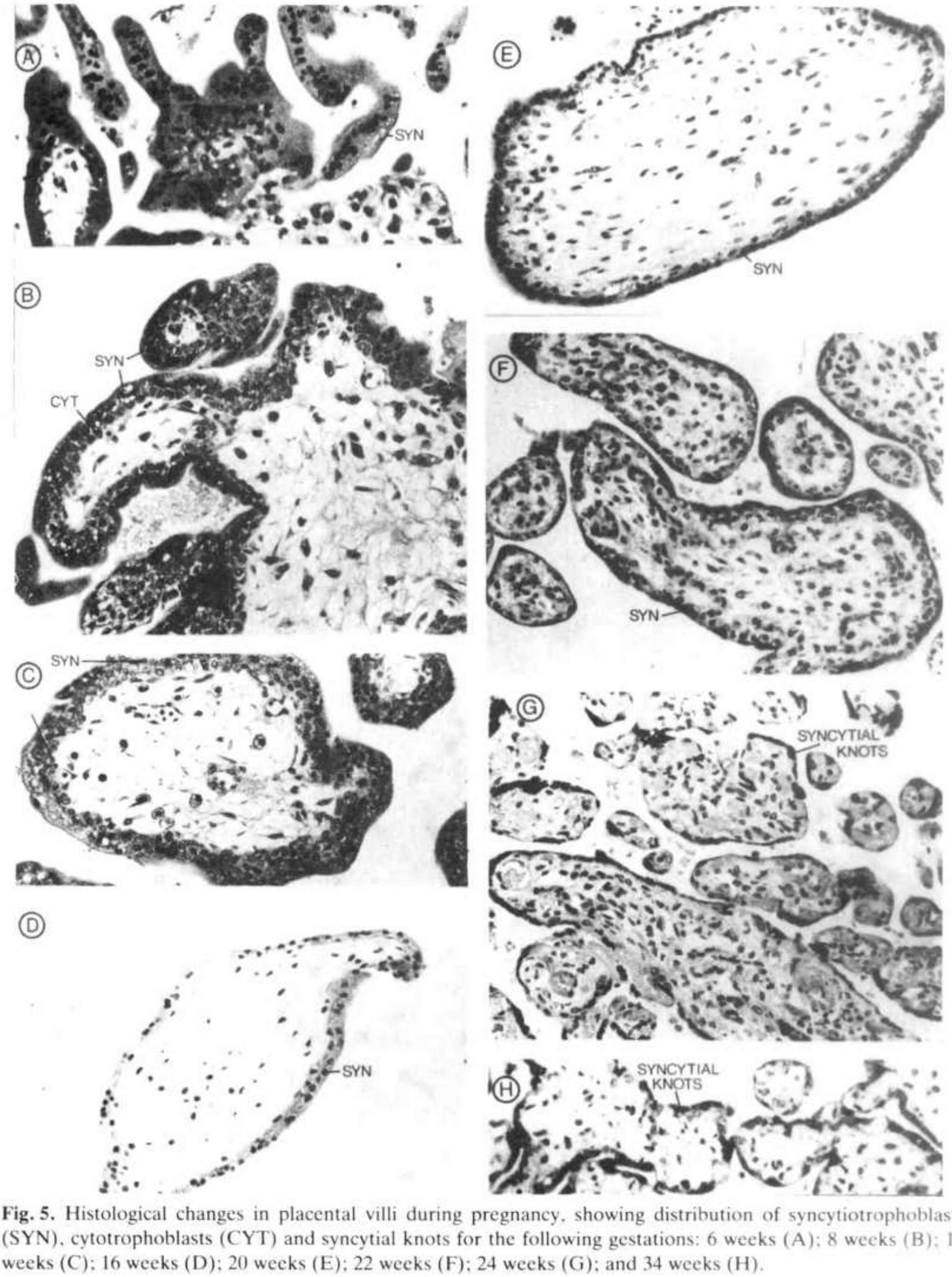

Fig. 5. Histological changes in placental villi during pregnancy, showing distribution of syncytiotrophoblasts (SYN), cytotrophoblasts (CYT) and syncytial knots for the following gestations: 6 weeks (A); 8 weeks (B); 10 weeks (C); 16 weeks (D); 20 weeks (E); 22 weeks (F); 24 weeks (G); and 34 weeks (H).

cytotrophoblasts in mature villi would cause diminished syncytium, reduced mRNA for both subunits and falling hCG levels. Also, rising αhCG secretion in late pregnancy would be linked to formation of syncytial knots in which activated cytotrophoblasts would accumulate αhCG mRNA. Such zones of cytotrophoblasts would secrete αhCG directly, and also introduce excess mRNA for αhCG over βhCG into the reduced syncytial regions. The small secondary rise in hCG observed in the third trimester would not be produced by mature villi but rather depend on proliferation of immature terminal villi. Increasing placental weight in late preg-

Table 1. Approximate width of placental syncytium and cytotrophoblast layers compared to hCG levels at various gestations

Gestation (weeks)	hCG median (i.u./l)	Syn. + cyt. (μm) Mean (range)	Syncytium (μm) Mean (range)	Average ratio $\frac{\text{Syn. + cyt.}}{\text{Syn.}}$	Placental number
4	1885	15 (5–37)	13 (5–35)	1·1	3
6	12 870	17 (5–35)	11 (4–27)	1·6	5
8	56 010	25 (6–42)	7 (3–14)	3·6	7
10	108 800	21 (6–37)	5 (2–13)	4·2	5
12	99 260	13 (4–30)	5 (2–8)	2·6	3
14	52 760	10 (4–25)	4 (2–7)	2·5	2
16	35 810	7 (2–18)	4 (2–7)	1·7	2
18	22 630	6 (2–12)	4 (2–7)	1·5	1
20	16 110	4 (2–12)	3 (2–5)	1·3	2

Syn., Syncytium; cyt., cytotrophoblast.

nancy depends on subdivision of immature terminal villi (Hamilton & Hamilton 1977). Thus the percentage of immature villi in the third trimester placenta would increase proportionately with the placental mass to cause rising hCG levels.

In early pregnancy, rising hCG levels coincide with a histology which reflects the invasive role of the developing placenta. Falling hCG levels after the first trimester coincide with a transformation in placental morphology and histology to enhance its role in feto-maternal transfer. Control of trophoblastic proliferation into maternal tissue is possible because the outer syncytial layer has a limited lifespan and is maintained by continual fusion from cytotrophoblasts (Ilgren 1983). By relating hCG mRNA levels to the processes that regulate syncytium formation, it is unnecessary to propose that hCG synthesis is controlled by activating genes in the syncytium. Instead, compounds reported to alter hCG production *in vivo* and *in vitro* (Hussa 1980) may act indirectly by altering the rate of cytotrophoblast differentiation. In the present study, the use of highly specific monoclonal antibodies enabled hCG, αhCG and βhCG profiles to be accurately monitored in maternal serum, and their profiles correlated to histological changes in the placenta. This correlation is explained by relating hCG production to the rate of cytotrophoblast differentiation.

Table 2. Comparison of median hCG levels and placental weights in late pregnancy*

Gestation (weeks)	No. of patients	hCG median (i.u./l)	Placental weight* (g) Median (range)
20	11	15 110	110 (70–180)
22	10	11 670	130 (80–190)
24	12	14 100	150 (80–220)
26	12	17 730	190 (85–280)
28	12	18 210	240 (90–410)
30	12	22 030	260 (130–510)
32	14	20 200	280 (100–560)
34	15	22 900	300 (110–600)
36	15	25 900	320 (110–650)
38	13	21 400	320 (110–620)

* Compiled from data presented by Hamilton & Hamilton (1977) and Braunstein *et al* (1980).

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The effect of sublingual nifedipine on uteroplacental blood flow in hypertensive pregnancy

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Summary. The effect of nifedipine on uteroplacental blood flow was investigated in nine hypertensive women in the third trimester of pregnancy and compared with the effects of a placebo in nine similar hypertensive women. An index of uteroplacental blood flow was obtained, twice before treatment and once after treatment, by measuring the increase in radioactivity in the region of the placenta with a gamma camera following an intravenous injection of indium-113m. There was no significant change in the blood flow index in either the nifedipine- or the placebo-treated groups despite a significant fall in blood pressure with nifedipine. Nifedipine lowers the blood pressure without any apparent reduction in uteroplacental blood flow.

The treatment of severe hypertension in pregnancy is a problem because antihypertensive agents sometimes produce side-effects and because the effect of these agents on uteroplacental blood flow is uncertain. The most commonly used drug for the treatment of severe hypertension is intramuscular or intravenous hydralazine which often causes side-effects such as headache, palpitations and tachycardia, although it does not significantly increase or decrease uteroplacental blood flow (Lunell *et al.* 1983). Nifedipine, a slow calcium channel blocker, is a potent antihypertensive agent with few side-effects (Haft & Litterer 1984) which acts rapidly when given sublingually (Frishman & Charlap 1984). When given orally in pregnancy hypertension it has been shown to be an effective antihypertensive agent with minimal side-effects (Walters & Redman 1984). Its effect on uteroplacental blood flow, however, is unknown. In hypertension in pregnancy the uteroplacental blood flow is often greatly decreased (Lunell *et al.* 1982, 1984) and drugs used for the treatment of hypertension in pregnancy should ideally produce a preferential vasodilatation of the uterine vessels and thereby increase the uteroplacental blood flow to normal levels. If the blood pressure is lowered and the perfusion pressure of the uterine circulation is reduced without preferential or concomitant vasodilatation of the uterine arteries and arterioles then uteroplacental blood flow will fall and the fetus may be compromised. This study was undertaken to determine the effect of a single sublingual dose of nifedipine on blood pressure and on uteroplacental blood flow with a view to its use in the emergency treatment of severe hypertension in pregnancy.

Patients and methods

Eighteen patients in the last trimester of pregnancy with hypertension with or without proteinuria were allocated to either a nifedipine

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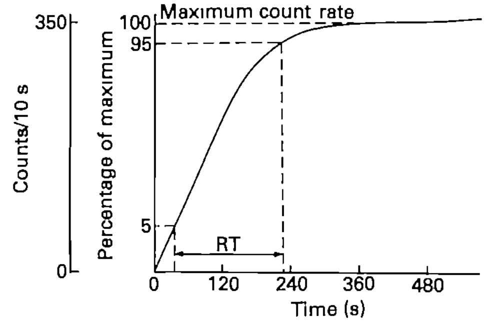

Fig. 1. Time activity curve over placental site following intravenous injection of indium-113m. RT, Rise time (time in seconds from the 5% to the 95% value of the maximum count rate).

or a placebo group by reference to the last digit of the hospital number which is allocated by a computer, patients with even end-digits being allocated to nifedipine and those with odd end-digits to placebo. Fifteen women were Cape Coloured and three were Black (Xhosa speaking) and the findings in the individual women were documented. Hypertension was defined as an average diastolic blood pressure of ⩾100 mmHg calculated from the mean of four 6-hourly readings taken whilst the women were resting in hospital the day before the study. Women with an anteriorly situated placenta were selected on ultrasound screening, and antenatal cardiotocography was performed in every woman to ensure that the fetal condition was not impaired. None of the women was in labour or had obvious uterine contractions and none had received an antihypertensive or any other medication likely to affect the blood pressure for at least 48 h. All the women were fully informed of the nature of the investigation and gave their written consent.

The women rested comfortably with 30° lateral tilt and a gamma camera was carefully positioned directly over the placenta as determined by ultrasound localization. The blood pressure and pulse rate were measured with an automatic blood pressure recorder (Spacelabs) at 5-min intervals. The women maintained a constant position and constant geometry in relation to the gamma camera, and the repeated blood flow measurements were performed with a standardized technique. Indium-113m in a dose of 37 MBq (1 mCi) was obtained from an indium-113m generator (Amersham International plc) and was injected into an antecubital fossa vein over 5 s. The indium-113m immediately binds to and labels plasma transferrin with a negligible amount of free indium. The increase in radioactivity of the blood entering the placenta and uterus was recorded with an Elscint 400 gamma camera with a medium-energy parallel-hole collimator interfaced to an A2 data processor for acquisition of serial summed images comprising 48 frames of 10 s each.

Three measurements were performed by repeat injections of indium-113m at 0, 30 and 60 min, and a sublingual dose of either nifedipine 5 mg or a placebo was given immediately after the second measurement.

On completion of the three measurements the summed images were examined and the placental site was identified by the region of maximum counts and was outlined as the region of interest using a graphic pen. The counts from the placental site were plotted against time and a time activity curve drawn for each blood flow measurement. Three indices were derived from each time activity curve (Fig. 1) as described by Lunell *et al.* (1979).

(i) *Rise time.* This is the time (in seconds) taken for the count rate to increase from 5% to 95% of the maximum value and is a measure of the rate of entry of blood into the choriodecidual space and adjacent areas of the uterus.

(ii) *Maximum count rate.* This is derived from the plateau of the time activity curve (counts/10 s) and is a measure of the total amount of blood in the region of the placental site.

(iii) *Uteroplacental blood flow index.* This is calculated from the maximum count rate divided by the rise time and is expressed as counts/s.

The blood flow index provides a measure of uteroplacental blood flow which is independent of the size of the placental blood pool. This is important as for the same flow rate a large placenta with a large placental blood pool will take longer to fill and to reach a maximum count rate

Table 1. Maternal and fetal characteristics in the two groups treated with nifedipine or placebo

	Nifedipine ($n = 9$)	Placebo ($n = 9$)
Mean age (years)	27·2	28·6
Proteinuria (no. of patients)	5	3
Median parity (range)	2 (0–5)	0 (0–4)
Mean gestational age (weeks)	35·8	34·7
Mean birthweight (g)	2313	2894
Mean placental weight (g)	486	550

Table 2. Blood pressure and pulse rate measurements before and after sublingual administration of nifedipine or placebo

	Median blood pressure (mmHg)						Median pulse rate (beats/min)	
	Systolic		Diastolic		Mean arterial			
Patient group	Before	After	Before	After	Before	After	Before	After
Nifedipine								
1	151	145	96	89	114	105	84	81
2	141	119	97	83	116	96	90	96
3	155	153	100	102	115	112	105	119
4	141	140	98	94	113	108	87	105
5	150	155	99	103	116	121	78	95
6	158	156	109	106	123	123	85	93
7	154	150	113	105	127	120	87	107
8	149	133	107	99	120	110	63	85
9	138	128	98	91	110	101	78	81
Mean	148·6	142·1	101·9	96·9	117·1	110·7	84·1	95·8
Placebo								
10	160	175	99	99	115	108	78	83
11	167	177	114	119	130	132	111	110
12	147	140	97	93	115	112	88	75
13	143	141	89	93	105	104	83	80
14	168	169	110	113	127	130	76	80
15	154	150	111	110	125	125	80	86
16	147	152	94	98	112	117	98	90
17	138	140	98	102	109	114	85	88
18	128	128	92	96	103	104	80	73
Mean	150·2	152·4	100·4	102·6	113·9	116·2	86·6	85

than a small placenta with a small placental blood pool. Measurements of rise time are therefore probably a valid measure of changes in uteroplacental blood flow in the same subject but cannot be used to compare blood flow between subjects. The validity of transferrin-bound indium-113m uptake as an index of uteroplacental blood flow and its relation to other methods of measurement has been reviewed by Lunell & Sarby (1979). The median values for the systolic, diastolic and mean arterial blood pressures and pulse rate were calculated and the differences before and after the placebo or nifedipine were analysed by paired *t*-tests. The rise time, maximum count rate and uteroplacental blood flow index were calculated and the differences between the measurements at 0, 30 and 60 min in the nifedipine- and placebo-treated groups of women were evaluated by analysis of variance.

The total dose of radioisotope given to each patient was 111 MBq (3 mCi). Indium-113 has a half-life of 99·5 min, binds tightly to plasma

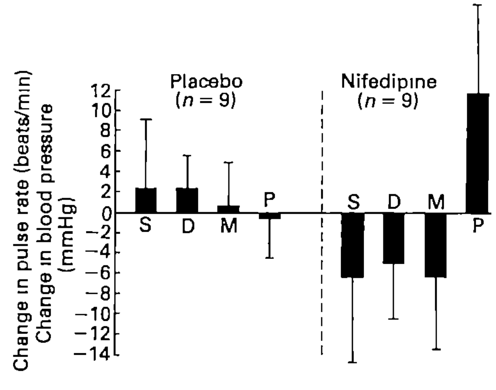

Fig. 2. Mean (SD) changes in systolic (S), diastolic (D) and mean arterial (M) blood pressure (mmHg) and pulse rate (P) (beats/min) observed after treatment with placebo or nifedipine. Significance of difference between haemodynamic changes after placebo and those after nifedipine: systolic ($P<0{\cdot}02$); diastolic ($P<0{\cdot}01$); mean arterial ($P<0{\cdot}02$); pulse rate ($P<0{\cdot}005$)

Table 3. Uteroplacental blood flow indices before (two observations) and after sublingual administration of nifedipine or placebo*

	Maximum count rate (counts/10 s)			Rise time (s)			Blood flow index (counts/s)		
	Before			Before			Before		
Patient group	1st	2nd	After	1st	2nd	After	1st	2nd	After
Nifedipine									
1	300	320	300	47	47	64	6·4	6·8	4·7
2	120	155	165	71	140	121	1·7	1·1	1·4
3	380	340	470	115	68	103	3·3	5·0	4·6
4	290	330	340	51	60	86	5·7	5·5	4·0
5	130	100	185	44	60	75	3·0	1·7	2·5
6	270	250	360	120	102	99	2·3	2·5	3·6
7	205	195	225	70	51	70	2·9	3·8	3·2
8	140	185	180	150	144	92	0·9	1·3	2·0
9	120	125	205	74	68	112	1·6	1·8	1·8
Mean	217·2	222·2	270	82·4	82·2	91·3	3·1	3·3	3·1
Placebo									
10	210	270	135	66	86	140	3·2	3·1	1·0
11	180	175	170	34	42	114	5·3	4·2	1·5
12	165	175	175	70	82	79	2·4	2·1	2·2
13	160	140	210	78	48	114	2·1	2·9	1·8
14	175	170	210	70	83	79	2·5	2·0	2·7
15†	225	—	350	97	—	112	2·3	—	3·1
16	190	155	205	122	139	73	1·7	1·1	2·8
17	160	185	205	82	75	69	2·0	2·5	3·0
18	340	300	410	49	50	50	6·9	6·0	8·1
Mean	200·6	196·3	228·9	74·2	75·6	92·2	3·2	3·0	2·9

*Indices of blood flow were measured after repeat injections of indium-113m, twice before treatment and once 30 min after treatment.

†Only one measurement before treatment.

transferrin and is not transferred to the fetus. The average whole-body radiation dose for the three blood flow measurements with the 111 MBq (3 mCi) dose is 0·51 mGy (51 mrad) to the mother and 0·24 mGy to the fetus (van der Merwe *et al.* 1970) which is well within the limits set by the International Commission on Radiological Protection Publication No. 26. This study was approved by the Groote Schuur Hospital radiation protection officer and by the ethics and research committee of the University of Cape Town Medical School.

Results

There was no significant difference in the characteristics of the women between the nifedipine- and placebo-treated groups (Table 1). All the women were of Cape Coloured origin except subject no. 1 in the nifedipine group and subjects nos 14 and 18 in the placebo group who were Black (Xhosa speaking). The median blood pressure and pulse rate measurements before and after treatment are shown in Table 2. There was a significant fall in the systolic, diastolic and mean arterial blood pressure and a significant rise in pulse rate after nifedipine but not after placebo (Fig. 2). The rise time, maximum count rate and blood flow indices at 0, 30 and 60 min in the placebo- and nifedipine-treated women are shown in Table 3. There were no significant differences between the 0- and 30-min baseline pretreatment observations or between these observations and the 60-min post-treatment observations after nifedipine or after placebo. The correlation coefficient of the first and second pretreatment blood flow indices in the whole series was 0·90 (Fig. 3) indicating that under the conditions of the present study the measurements of uteroplacental blood flow using indium-113m were reproducible and that the method provides a reliable means of assess-

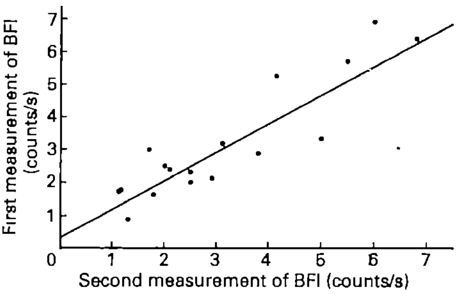

Fig. 3. Correlation between the first and second basal measurements, before treatment, of the utero-placental blood flow index (BFI) in 17 women (correlation coefficient = 0·90).

ing the acute effect of antihypertensive drugs on uteroplacental blood flow. The only maternal side-effects noted were a cutaneous flush and headache in one patient, and there was no abnormality in any of the fetal heart rate recordings throughout the study.

Discussion

This is the first report of the effect of nifedipine on uteroplacental blood flow in human pregnancy. Nifedipine has been shown to increase uterine blood flow in non-pregnant, non-oestradiol-treated rats but not in rats treated with oestradiol (Sandahl *et al.* 1978). In pregnant goats nifedipine produces a fall in blood pressure but no change in uterine blood flow as measured by an electromagnetic flow probe (Veille *et al.* 1986). Nifedipine has, however, been shown to cause vasodilatation of human umbilical arteries *in vitro* suggesting that nifedipine reduces vascular resistance in the fetal placental circulation (Maigaard *et al.* 1984). The present study indicates that following a sublingual dose of nifedipine the uteroplacental blood flow index is maintained in spite of a lowered arterial blood pressure and a lowered uterine artery perfusing pressure. This suggests that following administration of nifedipine there is a relative uterine vasodilatation and a relative decrease in uterine vascular resistance which is proportional to the fall in blood pressure. Nifedipine, however, does not appear to have a preferential effect on uterine vascular resistance or to preferentially increase uteroplacental blood flow.

Nifedipine is an effective and safe antihypertensive agent in the treatment of pregnancy hypertension as it produces a rapid smooth fall in blood pressure with minimal side-effects and does not appear to decrease the uteroplacental blood flow or impair fetal well-being. As nifedipine is rapidly absorbed when given sublingually and produces a fall in blood pressure within 5–10 min it is particularly useful in the treatment of severe hypertension in pregnancy when a rapid but controlled lowering of blood pressure is required. The maternal side-effects are minimal, consisting of occasional mild flushing and headache, and no adverse fetal effects have been reported.

Nifedipine has a number of other actions in addition to lowering the blood pressure which may be advantageous in the treatment of severe hypertension in pregnancy. It has been shown to produce a preferential dilation of the cerebral arteries and arterioles (Payen *et al.* 1984) which may be of benefit in eclampsia or imminent eclampsia where cerebral ischaemia is one of the main pathological features (Richards *et al.* 1986). Renal blood flow is also maintained after nifedipine administration in spite of a lowered blood pressure in both normal and hypertensive subjects (Bellini *et al* 1984). Furthermore, nifedipine has been shown to inhibit platelet aggregation and thromboxane synthesis (Dale *et al.* 1983) which may be an advantage in severe gestational proteinuric hypertension where acute or chronic disseminated intravascular coagulation is sometimes a major feature. Nifedipine is a potent antihypertensive drug which acts rapidly, does not cause any apparent reduction in the uteroplacental blood flow index, and may have additional beneficial therapeutic effects which make it a good drug for the acute treatment of severe hypertension in pregnancy.

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Blood flow velocity waveforms in large maternal and uterine vessels throughout pregnancy and postpartum: a longitudinal study using Duplex sonography

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Summary. Blood flow velocity waveforms in large maternal and uterine vessels were measured longitudinally from 16 weeks gestation onwards until 12 weeks postpartum in 21 singleton pregnancies by duplex sonography. In the maternal carotid artery, time average mean velocity (TAVmean) did not show significant changes. In both the femoral artery and vein, however, significant changes were observed. In the femoral artery, TAVmean and systolic maximum velocities decreased with advancing gestation. In the femoral vein, TAVmean remained constant throughout pregnancy and was lower than postpartum. The resistance index in the uterine arteries decreased with advancing gestation and increased after delivery. Among many factors contributing to femoral arterial blood flow velocity changes in pregnancy, we suggest that a major one is the increase in uterine blood flow. Reduction in venous femoral blood flow velocity and increase in the femoral vein diameter might be associated with the common occurrence of venous disorders in pregnancy.

Numerous studies have investigated maternal central cardiovascular haemodynamics in pregnancy. Though different measurement methods have been used, and though many results are controversial, it is generally agreed that cardiac output increases by 30–40% and then decreases—depending on the maternal position—at the end of pregnancy (Atkins *et al* 1981; Bader *et al.* 1955; Hamilton 1949; Lees *et al.* 1967; Mashini *et al.* 1987; McLennan *et al.* 1987; Rovinsky & Jaffin 1966; Ueland *et al.* 1969; Walters *et al.* 1966). Cardiac output increases by increasing heart rate and stroke volume. Since the increase in cardiac output does not lead to an increase in systemic blood pressure, peripheral resistance must decrease (Atkins *et al.* 1981; Bader *et al.* 1955; Brehm & Kindling 1955; Rovinsky & Jaffin 1966).

Many cardiovascular changes cannot be attributed solely to the increase in uterine blood flow and fetal needs. The rise in cardiac output occurs very early in the first trimester (Atkins *et al.* 1981; Lees *et al.* 1967; McLennan *et al.* 1987; Walters *et al.* 1966) and exceeds the increase in

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maternal oxygen consumption, which is about 10–20% at term (Bader *et al.* 1955). The total blood volume rises from 4000 ml to 5500 ml at 40 weeks gestation (Hytten 1985). The increased cardiac output is not only distributed to the uterus (Assali *et al.* 1953; Romney *et al.* 1955), but increasingly to the kidneys and the skin (de Swiet 1980; Katz & Sokal 1980).

In contrast to current knowledge about central vascular dynamics, our understanding of the regulatory function of the peripheral vascular system in pregnancy is incomplete. It is known that venous distensibility and capacity (Clarke-Pearson & Jelovsek 1981; Goodrich & Wood 1964) as well as femoral venous pressure are increased (McLennan 1943). Femoral venous blood flow is reduced (Ikard *et al.* 1971; Schlunk & Goeltner 1974) and venous return diminished both in the supine position and during quiet standing in late pregnancy (Kerr *et al.* 1964; Schneider *et al.* 1984). For the arterial system of the lower part of the body, a reduction in the velocity of the arterial pulse wave (Brehm & Kindling 1955), a decrease in mean circulation time (arm-to-thigh) (Rovinsky & Jaffin 1966) and in arterial flow in late pregnancy has been described (Bieniarz *et al.* 1968; Drummond *et al.* 1974).

In the past decade, Doppler examinations have been widely used for non-invasive estimation of fetal, uteroplacental and adult blood flow conditions (Gosling & King 1975; Griffin *et al.* 1983; Kirkinen *et al.* 1987; Trudinger *et al.* 1985). Duplex sonography, which allows the simultaneous recording of B-mode imaging and Doppler measurements, offers new possibilities in non-invasive diagnostics of the peripheral vascular system (Jaeger *et al.* 1985). The aim of this study was to measure blood flow velocity and waveform changes in large maternal and uterine vessels throughout pregnancy in order to attain a better understanding of maternal central and peripheral vascular dynamics.

Patients and methods

Table 1 gives details of the 21 study patients. All women were healthy non-smokers, without cardiovascular disease, and gave informed consent for the study. Nineteen pregnancies were uncomplicated and received only routine supplements of iron, fluoride, magnesium and vitamins. Two pregnancies were complicated by premature labour at 30 weeks gestation and were treated with betamimetics. These patients were excluded from the study during the time of this treatment. All 21 women gave birth to healthy children at between 36 and 41 weeks (mean 38·9, SD 1·4). Apgar scores at 5 min were between 8 and 10, pH of umbilical artery blood was between 7·18 and 7·38 (median 7·29) and all birthweights were above the 10th centile (mean 3405, SD 365, range 2650–4140 g).

The Doppler measurements were performed at 16, 20, 24, 28, 32, 36 and 38 weeks gestation and at 1, 6 and 12 weeks postpartum with the woman in a right semi-recumbent position (the upper body raised 30–40°). No woman complained about symptoms suggesting a vena cava syndrome.

The Doppler measurements were made with an ultrasound duplex system (Acuson Model 128, Acuson Computed Sonography, Mountain View, CA, USA). This duplex system combines B-mode imaging (linear and sector probes) and the bi-directional pulsed Doppler technique with real-time spectral analysis (fast Fourier Transform at 180 spectra/s, frequency resolution

Table 1. Characteristics of women in the study

	n	Mean (SD)	Range
Number	21		
Nulliparous	9		
Parous	12		
Age (years)		29·8 (6·2)	18–43
Height (cm)		164·3 (8·2)	152–178
Prepregnancy weight (kg)		56 8 (8·8)	45–82
Length of gestation (weeks)		38·9 (1·4)	36–41
Mode of delivery			
Vaginal	18		
Caesarean	3		

50 Hz). Acuson 128 computes blood flow velocity (m/s) versus time. The software gives instantaneous maximum and mean velocities at any time. From the available software, time average mean velocity (TAVmean) and the resistance index (RI) (maximum systolic flow velocity minus maximum end-diastolic flow velocity divided by the maximum systolic flow velocity) (Pourcelot 1974) were used for the study.

The large maternal vessels, carotid artery, femoral artery and vein, were examined with a 5-MHz linear probe, the uterine vessels with a 3·5-MHz probe. Doppler frequency in both probes is 3·5 MHz. For all measurements a high pass filter of 125 Hz was used. For the Doppler measurements in the femoral artery, the sample volume was placed in the straight part of the right femoral artery, immediately distal to the iliac artery. The carotid measurements were performed in the left common carotid artery. For blood flow velocity waveform analysis (Fig. 1), a small sample volume (1·5–2 mm) was located in the centre of the vessel. The maximum velocities were calculated over an average of four to five cardiac cycles. TAVmean was calculated over an average of four to five cardiac cycles with a sample volume covering the whole vessel.

The femoral vein can easily be identified medial to the femoral artery. Special care has to be taken not to compress the vein. The sample volume—covering the whole vessel—was located in the straight part of the vein. TAVmean was analysed over a time of 6–8 s. Measurements of the venous circulation started only at 20 weeks gestation.

The diameters of the large maternal vessels were measured from several randomly made B-mode images of the vessel's cross-section in the longitudinal scan.

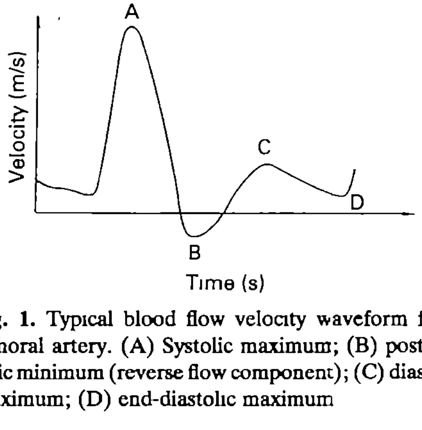

Fig. 1. Typical blood flow velocity waveform for a femoral artery. (A) Systolic maximum; (B) post-systolic minimum (reverse flow component); (C) diastolic maximum; (D) end-diastolic maximum

From the uterine vessels, the mean value of the resistance index (RI) was calculated over an average of five cardiac cycles. Throughout pregnancy, the uterine vessels (arcuate arteries) were examined on the placental side.

At every examination, maternal haematocrit, heart rate and blood pressure were determined. Blood pressure was measured indirectly on the left arm (Sphygmomanometer, Ruegge Medical, Baden, Switzerland). The mean of five values of systolic and diastolic pressures and of maternal heart rate were taken as the final values.

All Doppler recordings were stored on videotape for further analysis. The data were transferred into a database system. The results are presented as tables with the median and the 10th centile/90th centile and box plots (Chatfield 1983). The horizontal lines of the box show the 25th, 50th and 75th centiles; extreme values are plotted separately as open circles. Differences of values—significance tests were performed only by comparing values from 16 weeks and 38 weeks gestation and between 38 weeks gestation and 12 weeks postpartum—were tested using the Wilcoxon signed-rank test. A *P* value $<0{\cdot}05$ was considered significant.

Results

Maternal carotid artery

Table 2 summarizes the results of maternal carotid artery measurements. Throughout pregnancy, TAVmean and vessel diameter showed no significant changes, whereas maximum systolic and end-diastolic flow velocities decreased from 16 weeks gestation to 38 weeks gestation..

Femoral vein

TAVmean in the femoral vein was lower throughout pregnancy than postpartum (Fig. 2). The femoral vein diameter increased throughout pregnancy and is greater at any gestational age than in the non-pregnant state (Table 3).

Femoral artery

Table 4 represents the results of the femoral artery measurements. TAVmean decreased

Table 2. Time average mean velocity (TAVmean), systolic and end-diastolic maximum velocities and diameter of the common carotid artery during pregnancy and postpartum

	Weeks gestation							Weeks postpartum		
Carotid artery	16	20	24	28	32	36	38	1	6	12
TAVmean (m/s)	0·210 (0·134/0·272)	0·200 (0·138/0·270)	0·200 (0·140/0·300)	0·220 (0·178/0·230)	0·200 (0·154/0·255)	0·190 (0·141/0·249)	0·190 (0·152/0·250)	0·180 (0·150/0·256)	0·205 (0·170/0·268)	0·230 (0·163/0·294)
Systolic maximum velocity (m/s)	*1·125 (0·991/1·396)	1·126 (0·821/1·420)	1·026 (0·876/1·323)	1·030 (0·865/1·316)	1·083 (0·875/1·296)	0·937 (0·769/1·236)	*0·967 (0·730/1·169)	0·940 (0·756/1·108)	1·122 (0·910/1·279)	1·015 (0·830/1·220)
End-diastolic velocity (m/s)	*0·280 (0·198/0·316)	0·253 (0·196/0·326)	0·250 (0·195/0·325)	0·246 (0·191/0·363)	0·250 (0·187/0·349)	0·227 (0·182/0·303)	*0·235 (0·177/0·294)	0·203 (0·147/0·258)	0·250 (0·211/0·311)	0·275 (0·200/0·404)
Diameter (mm)	5·6 (4·6/6·8)	5·8 (5·3/6·7)	5·8 (5·3/6·6)	5·9 (4·8/6·8)	5·9 (5·1/6·9)	6·0 (5·1/6·7)	6·2 (5·0/6·9)	5·8 (5·1/6·8)	5·6 (4·5/6·9)	5·5 (4·8/6·0)

Results are shown as median (10th/90th centile) values

* $P<0.05$ values at 16 weeks compared with 38 weeks gestation

Table 3. Time average mean velocity (TAVmean) and diameter of femoral vein during pregnancy and postpartum

	Weeks gestation							Weeks postpartum		
Femoral vein	16	20	24	28	32	36	38	1	6	12
TAVmean (m/s)		0·057 (0·038/0·098)	0·061 (0·043/0·112)	0·055 (0·038/0·160)	0·050 (0·037/0·070)	0·058 (0·033/0·071)	0·052* (0·032/0·069)	0·130 (0·100/0·180)	0·140 (0·070/0·279)	0·160* (0·106/0·519)
Diameter (mm)		10·8 (9·0/11·9)	10·4 (8·4/11·3)	10·6 (7·0/12·5)	11·1 (9·5/13·6)	11·8 (10·5/13·6)	11·9* (10·1/15·0)	8·2 (5·5/12·5)	9·1 (4·5/13·1)	7·5* (1·3/10·9)

Results are shown as median (10th/90th centile) values

*$P<0.05$ values at 38 weeks gestation compared with 12 weeks postpartum

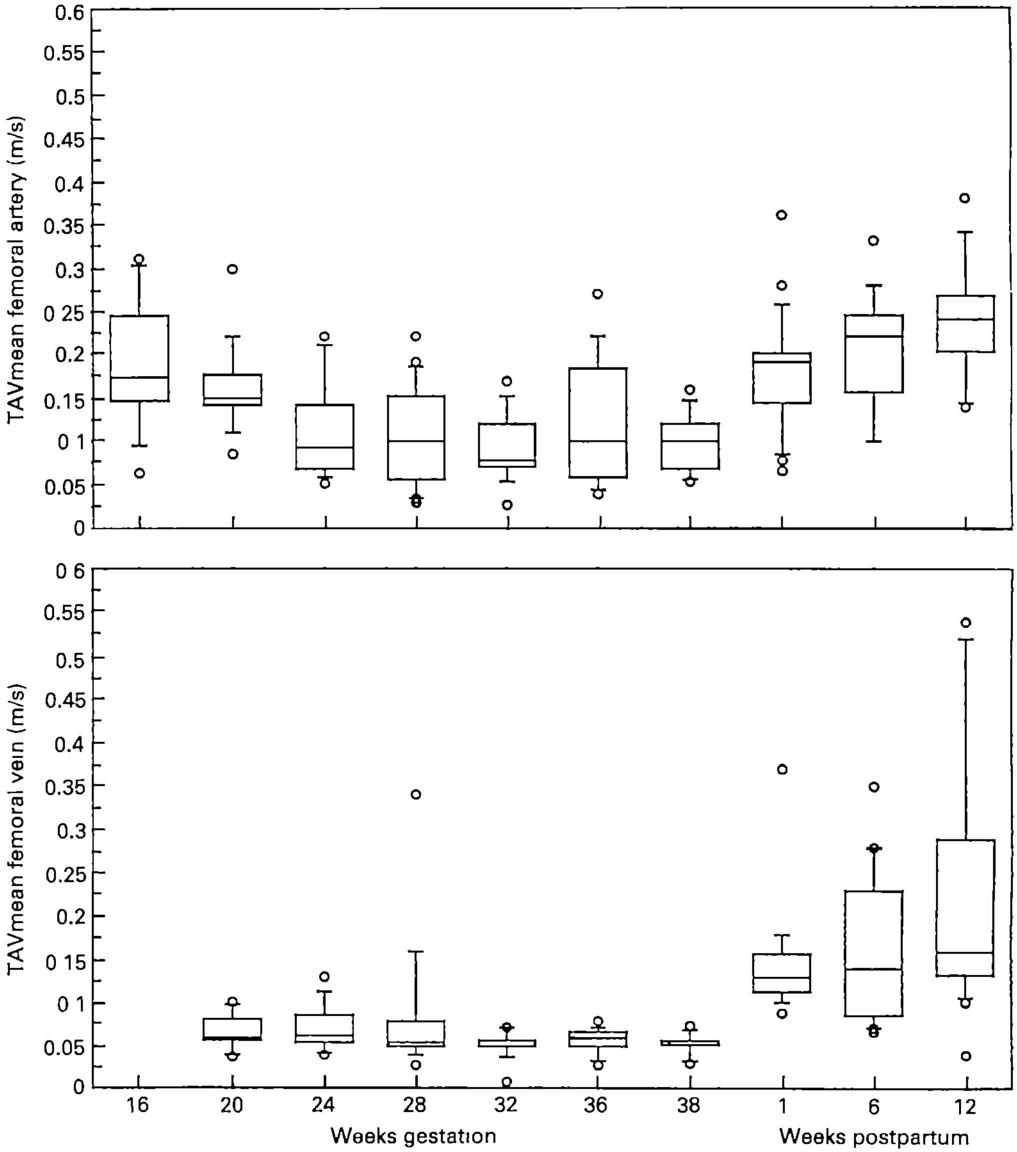

Fig. 2. Box plots with 25th, median, and 75th centiles of time average mean velocity (TAVmean) of the femoral artery and vein.

from 16 weeks to 38 weeks by about 45% (see also Fig. 2). Regarding the blood flow velocity waveform, systolic maximum velocity showed a significant decrease throughout pregnancy (Fig. 3). The increase in the femoral artery diameter (1 mm) was not statistically significant.

Uterine vessels

The RI in the arcuate arteries decreased throughout pregnancy from 46% at 16 weeks gestation to 36% at 38 weeks gestation (Fig. 4).

Blood pressure, maternal heart rate, haematocrit

Systolic and diastolic blood pressures have their lowest values in the second trimester. Median values of systolic blood pressure ranged between 104 and 111 mmHg; the diastolic values varied between 60 and 68 mmHg. Maternal heart rate reached a maximum at 32–36 weeks gestation (median value 89 beats/min) and dropped to 71 beats/min at 3 months postpartum. Haematocrit showed no significant changes throughout the

Table 4. Time average mean velocity (TAVmean), systolic maximum, post-systolic minimum and diastolic maximum velocities, and diameter of the femoral artery during pregnancy and postpartum

	Weeks gestation							Weeks postpartum		
Femoral artery	16	20	24	28	32	36	38	1	6	12
TAVmean (m/s)	*0·175 (0·096/0·303)	0·150 (0·110/0·220)	0·093 (0·060/0·210)	0·100 (0·035/0·186)	0·079 (0·053/0·152)	0·100 (0·043/0·220)	*0·100** (0·056/0·146)	0·190 (0·087/0·256)	0·220 (0·100/0·280)	0·240** (0·144/0·340)
Systolic maximum velocity (m/s)	*1·088 (0·851/1·403)	1·028 (0·779/1·314)	0·820 (0·656/1·118)	0·793 (0·619/1·110)	0·832 (0·626/1·026)	0·810 (0·610/1·077)	*0·805** (0·558/1·055)	0·973 (0·776/1·306)	1·025 (0·801/1·227)	1·130** (0·851/1·511)
Post-systolic minimum velocity (m/s)	−0·332 (−0·441/−0·251)	−0·324 (−0·420/−0·192)	−0·320 (−0·405/−0·243)	−0·378 (−0·441/−0·212)	−0·324 (−0·388/−0·252)	−0·330 (−0·420/−0·183)	−0·310** (−0·420/−0·240)	−0·250 (−0·369/−0·190)	−0·261 (−0·337/−0·138)	−0·207** (−0·301/−0·131)
Diastolic maximum velocity (m/s)	0·176 (0·138/0·280)	0·155 (0·078/0·207)	0·140 (0·010/0·160)	0·110 (0·065/0·162)	0·128 (0·086/0·168)	0·130 (0·069/0·187)	0·123** (0·098/0·173)	0·165 (0·123/0·236)	0·165 (0·113/0·243)	0·177** (0·121/0·252)
Diameter (mm)	6·4 (5·7/7·7)	7·1 (6·2/7·6)	7·3 (6·2/7·8)	6·9 (5·8/8·4)	7·5 (6·1/8·2)	7·5 (5·9/8·2)	7·5 (5·8/8·3)	6·9 (5·6/7·6)	6·7 (5·5/7·4)	6·6 (5·8/8·0)

Results are shown as median (10th/90th centile) values

* $P<0·05$ values at 16 weeks compared with 38 weeks gestation.

** $P<0·05$ values at 38 weeks gestation compared with 12 weeks postpartum.

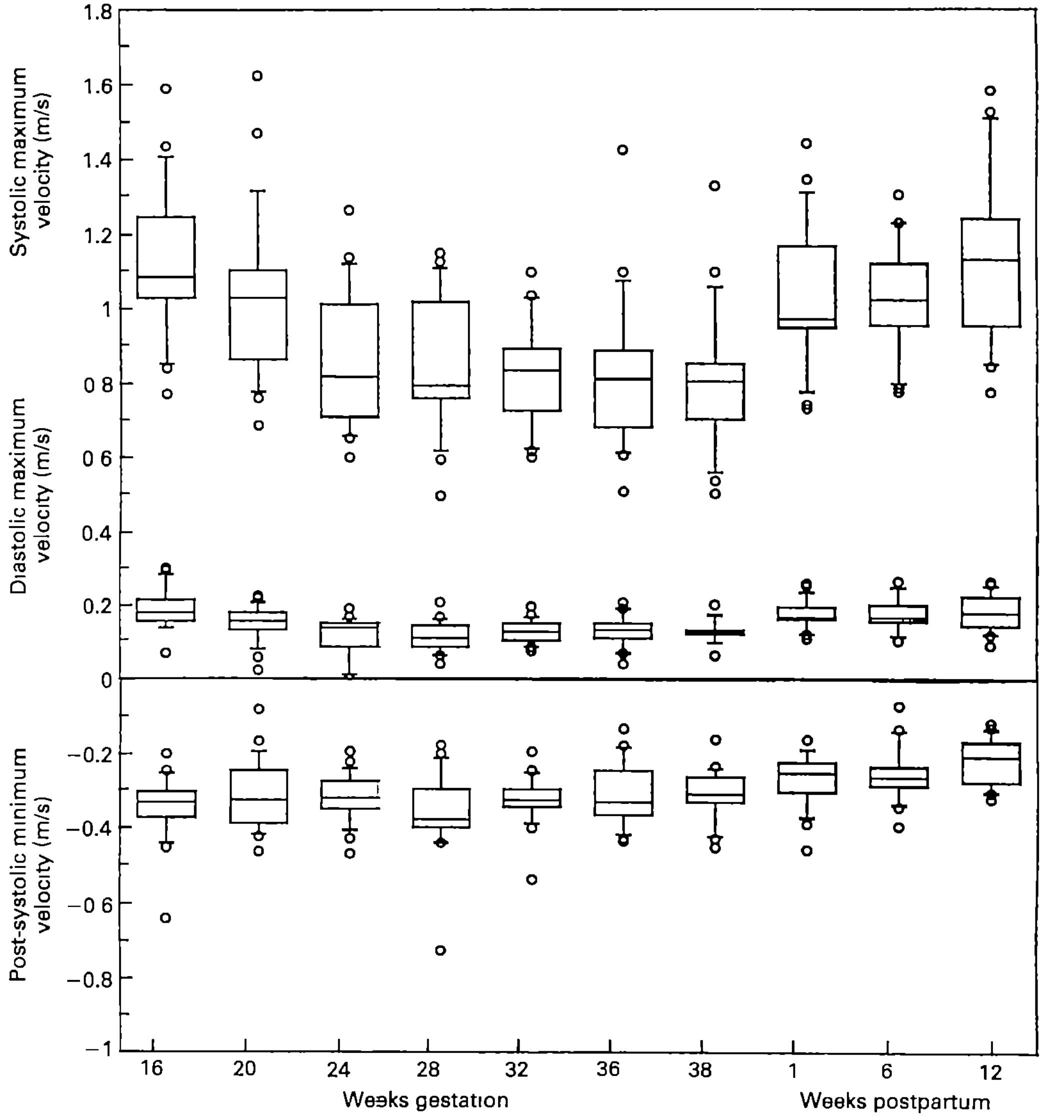

Fig. 3. Box plots of peak velocities in the femoral artery.

study period (median: 35·8% at 16 weeks, 36·8% at 38 weeks and 36·7% at 12 weeks postpartum).

Discussion

It must be kept in mind that in the Doppler technique there are certain inherent sources of error in the estimation of blood flow, e.g. possible inaccuracy in measuring the vessel's cross-section, in adjusting the Doppler beam to the optimal angle, and in analysing the Doppler shift (Gill 1985; Griffin *et al.* 1983). Some of these limitations can be reduced by careful handling of the Doppler technique by the same examiner, by the use of a sophisticated Doppler system such as Acuson 128, which allows continuous and simultaneous recording of Doppler shift and real-time B-mode imaging and thus control of the correct position of the sample volume and the optimal angle of approach, and by a longitudinal study design, where inherent sources of error of the applied technique remain the same throughout a study and where relative changes of values are of greater interest than absolute values.

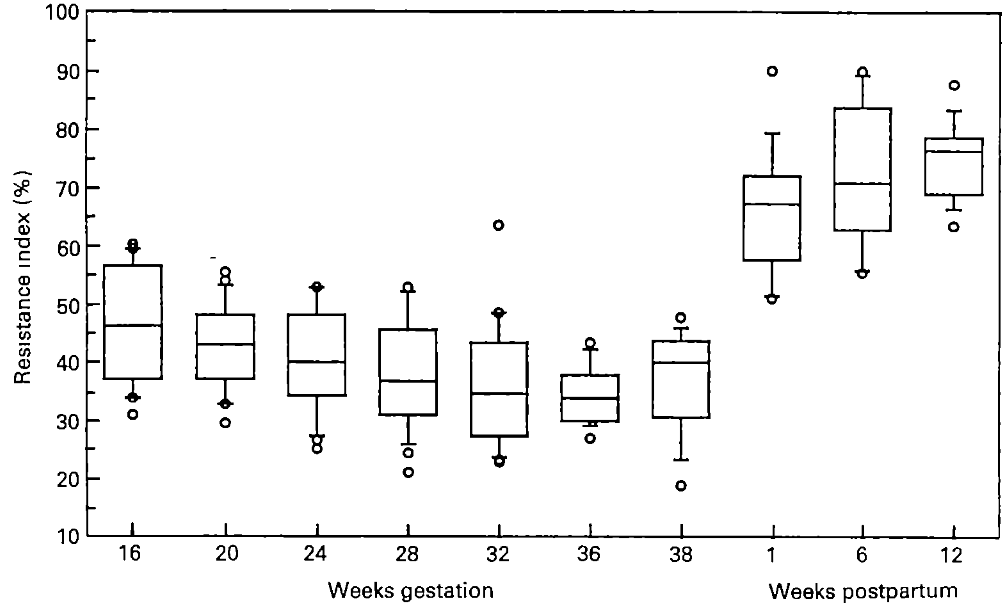

Fig. 4. Box plots of resistance index of uterine vessels.

The results of this study show significant blood flow velocity changes throughout pregnancy compared with the postpartum period in the femoral artery and femoral vein and, to a much smaller degree, in the common carotid artery. In the femoral artery, mean velocity decreases by about 45% and typical blood flow velocity waveforms show a decrease in the systolic maximum velocity. There are many factors to be considered which might contribute to alterations in blood flow velocity in the femoral artery (McDonald 1974): changes in pressure, in vessel diameter, in viscosity of blood, in visco-elastic properties of the vessel wall. In pregnancy, alterations of femoral blood flow velocity might also be caused by compression of the aorta by the growing uterus and the increasing blood supply to the uteroplacental circulation. Regarding our results concerning blood pressure and haematocrit, pressure or viscosity changes can be excluded as major factors. Changes in contractility of arteries in pregnancy have been described in animal experiments (McLaughlin & Keve 1986). Radiographic studies show an aortic compression by the growing uterus (Bieniarz *et al.* 1968; Ohlson 1978), so postural effects cannot be excluded, but are probably of minor importance in a semi-recumbent position.

Considering our observation of an increase in femoral diameter throughout pregnancy by 1 mm (though this increase is statistically not significant) and the fact that an increase in diameter is a major factor which may lead to a reduction of mean blood flow velocity, there arises the question of the accuracy of our measurements of vessel diameter. At the beginning of the study, we did not have the M-mode technique, so we had to measure the vessel's diameter by the B-mode technique. Inaccuracy derives from the degree of precision of the caliper, which is only 0·1 mm, and from possible radial dilatation of the vessel wall during the different phases of the cardiac cycle. It is known that radial dilatation during systole is rather small in the femoral artery (McDonald 1974). We could confirm this finding for pregnant women as well. Using the M-mode technique, we found a radial dilatation of only about 5% during systole compared to the diastole at different gestational ages. We assume that our diameter measurements with several randomly made B-mode images of the vessel's longitudinal scan always at the same place by the same examiner are quite accurate for the available technique. If there is an increase in femoral arterial diameter, this could be a factor reducing the mean blood flow velocity.

Our observation of a decrease in RI in the uterine vessels throughout pregnancy indicates a progressive decrease in uterine vascular resistance and is in agreement with other Doppler studies (Campbell *et al.* 1986; Trudinger *et al.* 1985). After delivery, there is a rapid increase in resistance in the uterine circulation. These changes in resistance and changes in blood flow to the uterus must be considered as the major factors contributing to blood flow velocity changes in the femoral artery.

There is little known about the effect of pregnancy upon circulation in the upper part of the body. Cerebral circulation seems to remain unchanged (McCall 1949). The reports on circulation times (arm-to-tongue, arm-to-lung) are very contradictory: Greenstein & Clahr (1937) found an increase, Cohen & Thomson (1936) and Manchester & Loube (1946) a slight decrease—but with values still within the range for non-pregnant adults—and Wright *et al.* (1950) found no change. The absence of significant changes in mean blood flow velocity in the carotid artery corresponds to the latter findings.

The reduction of mean blood flow velocity in the femoral vein is already present at the beginning of the second trimester. A reduction of venous blood flow velocity has been described in other studies. Wright *et al.* (1950), using radioisotopes, found an increase in the circulation time from the foot to the groin beginning in the second trimester. At term, flow velocity was reduced by half. Goodrich & Wood (1964) found a significant decrease in calf venous blood flow in the third trimester. Ikard *et al.* (1971) found by the Doppler technique a reduction in femoral venous blood flow with advancing gestation and in relation to the maternal position (most pronounced when standing). Schlunk & Goeltner (1974) observed a reduction in venous blood flow of 43% at term. Schneider *et al.* (1984) found during quiet standing in late pregnancy an intermittent reduction of femoral blood flow velocity in 65% of the patients.

Reduction in the venous blood flow velocity can be related to increasing venous distensibility and pressure and reduced arterial inflow. Venous distensibility increases throughout pregnancy (Clarke-Pearson & Jelovsek 1981; Goodrich & Wood 1964). This increase occurs very early in pregnancy due to decreased venous tone. Venous capacity can increase in pregnancy by up to 50%, with a marked rise in the first and second, and little increase in the last trimester (Clarke-Pearson & Jelovsek 1981). On the other hand, antecubital venous pressure measurements are not significantly different from the non-pregnant values and do not vary in pregnancy. Femoral venous pressure rises at the beginning of the second trimester and drops soon after delivery (McLennan 1943). In late pregnancy the vena cava is compressed by the growing uterus and venous return is reduced (Kerr *et al.* 1964).

The reduction in femoral venous blood flow velocity seen already in the early stages of pregnancy might be related to increasing distensibility and venous capacity, probably caused by hormonal factors. Later in pregnancy, venous compression, increased venous pressure and decreased arterial inflow might be other contributing factors. Venous disorders are common in pregnancy and our findings of reduced blood flow velocity in the femoral vein might be an associated factor (Sumner 1981).

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Immunohistological localization of pregnancy-associated endometrial α_2-globulin (α_2-PEG) in endometrial adenocarcinoma and effect of medroxyprogesterone acetate

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Summary. Endometrium from postmenopausal women with endometrial adenocarcinoma was examined immunohistochemically using a monoclonal antibody to pregnancy-associated endometrial α_2-globulin (α_2-PEG), the major secretory protein of the glandular epithelium during the late luteal phase of the menstrual cycle and early pregnancy. Specimens were obtained at initial diagnostic curettage and at hysterectomy after medroxyprogesterone acetate (MPA) therapy. α_2-PEG was not detected in any malignant tissue irrespective of histological differentiation. Non-malignant endometrium obtained in association with malignant tissue was negative for α_2-PEG before treatment although after MPA therapy all specimens obtained exhibited marked α_2-PEG localization in glands. In four specimens endogenous alkaline phosphatase was observed consistently only in the malignant endometrium. Malignant endometrium does not appear to synthesize α_2-PEG nor is its synthesis induced by an oral progestogen, so that it does not represent a useful marker for endometrial carcinoma. Non-malignant endometrium in postmenopausal women appears to be fully capable of α_2-PEG production after stimulation with an oral progestogen.

Pregnancy-associated endometrial α_2-globulin (α_2-PEG) is a dimeric glycoprotein which was identified as an endometrial secretory protein whose synthesis and secretion increases from the mid-luteal phase of the menstrual cycle (Bell *et al.* 1985, 1986). During the late luteal phase and early first trimester of pregnancy it represents the major secretory protein of the endometrium and appears to be the major product of the secretory glandular epithelium since it is principally localized in this cell type (Bell 1988*a*, *b*; Waites *et al.* 1988). It is apparent that α_2-PEG is identical to progestogen-dependent endometrial protein (Joshi *et al.* 1980; Joshi 1983) and placental protein 14 (Bohn *et al.* 1982; Bell & Bohn 1986; Julkenun *et al.* 1986) and recently N-terminal amino acid sequence analysis of α_2-PEG (Bell *et al.* 1987) and PP14 (Huhtala

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et al. 1987) has suggested it represents a human homologue of the milk whey protein, β-lactoglobulin. Although progesterone has no short-term effect on α_2-PEG secretion *in vitro* it is apparent that α_2-PEG production is associated with the progesterone-dependent histologically defined differentiation of the glandular epithelium and it is suggested that α_2-PEG serum levels reflect its development and function (Bell 1986; Bell & Smith 1988).

Endometrial carcinoma may be classified histologically according to the degree of differentiation exhibited (Hendrickson & Kempson 1980). However, no information is available to determine whether this is reflected in production of secretory products associated with differentiation of the normal endometrium. Progestogens are effective agents in the management of recurrent and advanced endometrial carcinoma with an objective response in 30–50% of patients (Kneale 1986). It appears that a better response is obtained with the more differentiated tumour (see Kneale 1986). Studies have also suggested that the presence of steroid receptors may serve as a marker for carcinomas with more favourable prognosis with progestogen therapy and be possessed by the more differentiated tumour (Soper & Christenen 1986; Quinn *et al.* 1985*a,b*). Since progestogens presumably act through the progesterone receptor, carcinomas responding to therapy may produce proteins whose synthesis is dependent upon steroid–receptor genomic interactions.

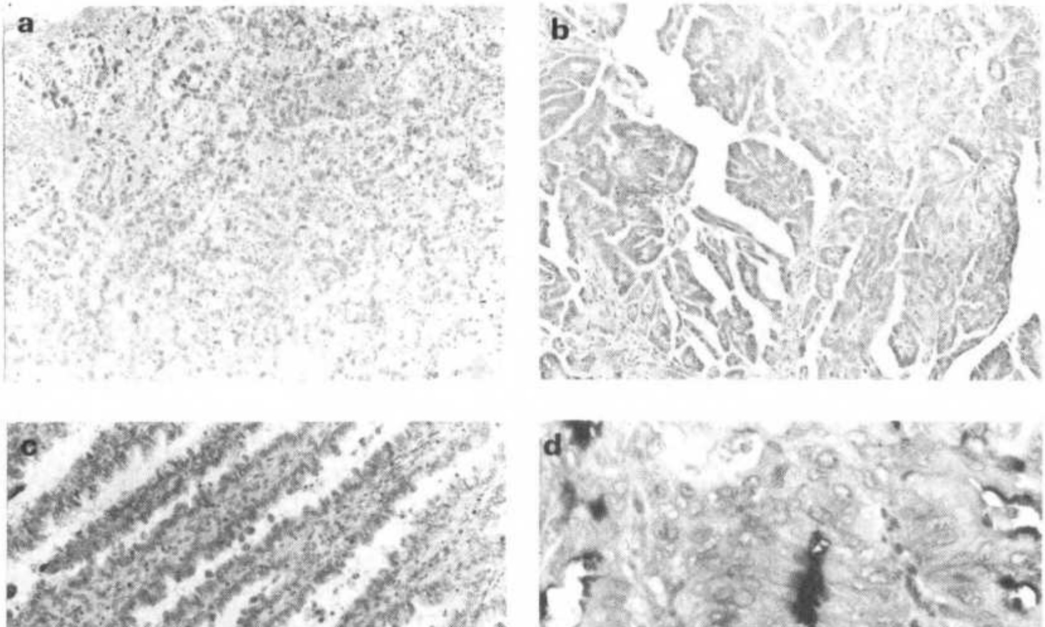
To determine whether α_2-PEG, a progesterone-dependent endometrial protein detectable in serum, could be employed as a marker for endometrial carcinoma or as a marker for progestogen-responsive carcinomas, the presence of this protein in carcinomas before and after progestogen therapy has been examined immunohistologically with a monoclonal antibody.

Patients and methods

This retrospective study included 16 women (mean age 65·5 years) with endometrial adenocarcinoma who presented with postmenopausal bleeding between 1984 and 1985. After a diagnostic curettage, all patients were given oral medroxyprogesterone acetate (MPA) (daily dosage 200–600 mg) for a mean duration of 2·93 weeks until definitive treatment, which comprised total abdominal hysterectomy and bilateral salpingo-oophorectomy in 15 women and radiotherapy only in one woman. Further curettings were obtained from this particular patient 21 weeks after starting MPA. Endometrial specimens from postmenopausal women, with no endometrial pathology, undergoing a vaginal hysterectomy for uterine prolapse were also obtained. All tissues were immersed in 10% neutral buffered formalin, and serial 5-μm paraffin sections were used for immunohistochemical staining. Haematoxylin and eosin stained sections were used for assessing the degree of tumour differentiation. The criteria of Hendrickson & Kempson (1980) were applied and all tissues were assessed by the same pathologist (R.A.W.).

Immunohistochemical procedure for α_2-PEG localization

A mouse monoclonal antibody was employed (code C6H11) (S. C. Bell, S. R. Patel, J. A. Jackson, K. Walczak & R. F. L. James, unpublished observations) as a culture supernatant at a routine dilution of 1:2. On selected specimens which possessed intense staining this was repeated with a concentration of 1:20. The staining involved an alkaline phosphatase-anti-alkaline phosphatase (APAAP) method (Cordell *et al.* 1984). All incubations were at room temperature with rocking in a moist chamber and all dilutions and washes done in Tris-buffered saline (TBS), pH 7·6, with 10% BSA. After dewaxing, rehydration and washing of sections, normal rabbit serum (Dako Ltd) was applied diluted 1:5 for 30 min to block non-specific binding. The primary monoclonal antibody to α_2-PEG was applied for 1 h, rabbit anti-mouse immunoglobulin antiserum (Dako Ltd) at a dilution of 1:50 for 30 min followed by mouse alkaline phosphatase-anti-alkaline phosphatase complex (Dako Ltd) at a dilution of 1:100 for 30 min. Alkaline phosphatase was developed for 1 h using Naphthol AS BI phosphoric acid and Fast Red in veronal acetate buffer, pH 9·2, containing 2 mM levamisole (Sigma, Poole, Dorset, UK). Sections were counterstained with Mayers' haematoxylin. Negative controls used were supernatants from NSO parent myeloma cell culture. Where these controls were positive a control was employed where sections were incubated with the substrate for alkaline phosphatase to detect the presence of endogenous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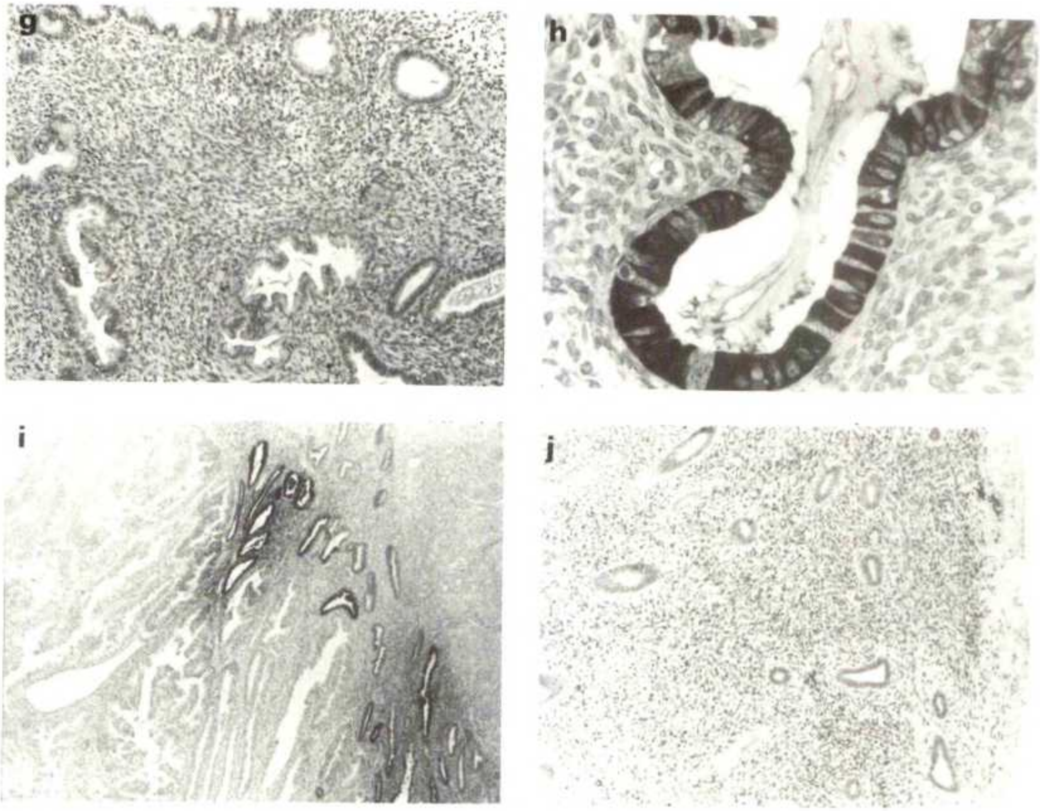

Fig. 1. Micrographs of specimens immunostained with monoclonal antibody against α_2-PEG using the APAAP method and counterstained with haematoxylin. (a) Poorly differentiated adenocarcinoma, pretreatment (curetting, patient 16). ×71. (b) Moderately differentiated adenocarcinoma, post-treatment (hysterectomy, patient 11). ×71. (c) Well differentiated adenocarcinoma, post-treatment (hysterectomy, patient 4). ×71. (d) Poorly differentiated adenocarcinoma, post-treatment (hysterectomy, patient 14). Note staining on apical cellular membranes for endogenous alkaline-phosphatase. ×286. (e) Poorly differentiated adenocarcinoma, pretreatment (curetting, patient 15). Note region of non-malignant endometrium adjacent to malignant endometrium (arrowed). ×71. (f) Poorly differentiated adenocarcinoma post-treatment (hysterectomy, patient 15). Note positive staining for α_2-PEG in endometrial glands in region of histologically non-malignant endometrium. ×71. (g) As for (f) except that supernatant from NSO parent myeloma cell culture was employed instead of monoclonal antibody. ×71. (h) As for (f) (patient 15) demonstrating intracellular localization of staining in glandular epithelium of histological non-malignant endometrium. ×286. (i) Moderately differentiated adenocarcinoma, post-treatment (hysterectomy, patient 9). Note positive staining for α_2-PEG in endometrial glands in region of histologically non-malignant endometrium. ×71. (j) Postmenopausal endometrium. ×71.

alkaline phosphatase not inhibited by levamisole.

Results

The 16 patients studied had a mean age of 65·5 years (range 48–86 years) and possessed endometrial adenocarcinoma which represented a range of poorly to well differentiated adenocarcinomas as histologically defined. Carcinoma tissue was obtained before treatment by curettage, and after treatment at hysterectomy in 15 women and by repeat curettage in the remaining woman after radiotherapy. Although hysterectomy after treatment enabled thorough examination of specimens for non-malignant endometrial tissue, the proportion of specimens with non-malignant tissue after treatment (7 of 16) was similar to that found in the curettage specimens obtained before treatment (6 of 16). The results are summarized in Table 1 and observations concerning malignant and non-malignant endometrium will be considered separately.

No specific immunostaining for α_2-PEG was observed in any malignant tissue either before or after MPA treatment whether poorly (Fig. 1a, e, f), moderately (Fig. 1b, i) or well differentiated (Fig. 1c). However, in malignant tissue of four patients (25%), one well differentiated, two moderately differentiated and one poorly differentiated, positive staining was observed which was demonstrated to be due to endogenous alkaline phosphatase activity. An identical staining pattern in these specimens was observed when sections were incubated in enzyme substrate alone, with monoclonal antibody and other reagents omitted. In three patients this activity was also demonstrated on malignant tissue after treatment. The endogenous alkaline phosphatase activity was principally localized to apical membranes of epithelial cells and glandular lumina when present (Fig. 1d). This alkaline phosphatase, not inhibited by levamisole, is thus of the type observed on the intestine and ovarian carcinomas.

The pattern of immunostaining for α_2-PEG in non-malignant endometrium was dramatically different. Of the six pretreatment specimens which contained non-malignant endometrium associated with the malignant tissue no specific staining was observed in four, and of the remaining two, only a single positive cell was detected in one and a cluster of positive cells was observed in the other. In those rare cells immunostaining was localized to the cytoplasm of glandular epithelial cells. To determine whether this non-malignant endometrium was affected by the

Table 1. Immunohistological localization of α_2-PEG in malignant and non-malignant endometrium before and after treatment with medroxyprogesterone acetate (MPA)

Patient	Age (years)	Tumour differentiation	MPA (mg/day)	Time (weeks)	Non-malignant endometrium		Malignant endometrium	
					Pre-MPA	Post-MPA	Pre-MPA	Post-MPA
1	62	Well	600	4	NP	NP	– (ES)	– (ES)
2	48	Well	600	2	NP	+	–	–
3	71	Well	300	1·5	NP	+	–	–
4	68	Well	300	3	NP	NP	–	–
5	50	Moderate	600	2	NP	NP	–	–
6	77	Moderate	400	1·5	–*	NP	–	–
7	86	Moderate	400	2	NP	NP	–	–
8	69	Moderate	400	2	NP	+	– (ES)	–
9	63	Moderate	300	4	–	+	–	–
10	64	Moderate	300	21(R)	–	NP	– (ES)	– (ES)
11	56	Moderate	200	4·5	–*	NP	–	–
12	67	Moderate	300	5	–	+	–	–
13	58	Poor	300	3·5	NP	NP	–	–
14	71	Poor	300	2	NP	NP	– (ES)	– (ES)
15	60	Poor	300	4	–	+	–	–
16	69	Poor	300	3	NP	+	–	–

NP, Tissue not present; ES, endogenous alkaline phosphatase; +, positive; –, negative; *, rare cell positive; R, radiotherapy.

presence of malignant endometrium, endometrium was obtained from postmenopausal women with no uterine or ovarian pathology. In these specimens no immunostaining for α_2-PEG was detected (Fig. 1j). In non-malignant endometrium, hormone treatment resulted in a marked effect upon α_2-PEG immunostaining. In all specimens after treatment where non-malignant endometrium was detected, intense immunostaining was observed in the endometrial glandular epithelium of most glands (Fig. 1f, i compared to e), which was localized to the cytoplasm of cells (Fig. 1h) and to glandular secretions. This pattern of staining was consistent regardless of whether the non-malignant endometrium was atrophic or whether it exhibited changes of either cystic or atypical hyperplasia. The staining represented specific staining for α_2-PEG as evidenced by negative staining in control sections (Fig. 1g).

Discussion

Immunohistological examination of endometrial adenocarcinoma specimens failed to demonstrate production of α_2-PEG, the quantitatively major secretory protein of the glandular epithelium under the influence of progesterone in normal endometrium (Bell 1986, 1988*a,b*). Treatment with the progestogen, MPA, also failed to induce the production of α_2-PEG. It has been reported that progesterone receptors can be detected in 59%, 60% and 82% of poorly, moderately and well differentiated endometrial carcinomas (Creasman *et al.* 1985). However, the absence of an α_2-PEG response in malignant tissue indicates that the presence of this receptor may not reflect the presence of a functional progesterone receptor mechanism if α_2-PEG gene expression is an ultimate effect of progesterone action. Other responses characteristic of progesterone action on the normal endometrium may be induced by progestogens in malignant tissue since histological alterations characteristic of progesterone action have been reported (Truskett 1965). This situation may be similar to that in hormone-dependent breast carcinomas where the production of the milk protein, α-lactalbumin, has not been detected (Hall *et al.* 1981). These observations suggested that α_2-PEG cannot be employed as a characteristic marker of endometrial carcinoma or response of this tissue to progestogens.

Nevertheless, an intriguing observation in this study was the detection of immunoreactive α_2-PEG in regions of non-malignant endometrium only after treatment with the progestogen. The absence of detection in non-malignant and postmenopausal endometrium suggests that the hormonal milieu in these patients does not induce α_2-PEG synthesis and reflects the suggestion of its progesterone dependence. Its appearance in the cytoplasm of glandular epithelium and glandular secretion after MPA therapy is identical to its cellular localization during the luteal phase of the menstrual cycle (Waites *et al.* 1988) and reflects the in-vivo progestogenic potency of MPA, which presumably in the glandular epithelium can bind to receptors and mediate steroid receptors' genomic regulation. It also implies that the postmenopausal endometrium is fully functionally competent in terms of its ability to produce the major secretory protein of the differentiated glandular epithelium. Whether peripheral serum levels of α_2-PEG would increase in patients after MPA treatment, thus reflecting the presence of non-malignant endometrium, remains to be determined, but the study does demonstrate that increases in serum levels of this protein in these patients could be mistaken for an MPA response by carcinoma.

Although the progesterone-dependent secretory endometrial protein α_2-PEG does not appear to be a useful marker for endometrial adenocarcinoma, it appears to be a useful marker to study the relative progestogen potency of progestogens on the endometrium.

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The binding of epidermal growth factor to the human uterus and leiomyomata in women rendered hypo-oestrogenic by continuous administration of an LHRH agonist

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Summary. The binding of epidermal growth factor (EGF) to human myometrium and leiomyomata was assessed in a group of women rendered hypo-oestrogenic with the LHRH agonist Zoladex (ICI 118630). The results were compared with those obtained with tissues from women with normal cycles. In normal women, the specific binding of radiolabelled [^{125}I] EGF to both myometrial and fibroid homogenates did not vary during the menstrual cycle, but the specific binding of [^{125}I] EGF to fibroid in women treated with LHRH agonist was significantly less than in the untreated group. Since the hypo-oestrogenic state induced by the agonist is associated with a decrease in fibroid size, the results suggest that the effect of oestrogen on fibroid tissue may partly be mediated by EGF.

Epidermal growth factor (EGF) is a member of the group of peptide hormones which control the rate and extent of cell proliferation. While studying nerve growth factor, Cohen (1960) identified an extract of mouse submaxillary gland which induces precocious opening of the eyelid and incisor eruption due to direct stimulation of epidermal growth and keratinization (Cohen & Carpenter 1975). This 53 amino acid peptide (Savage & Cohen 1972) is closely related in structure and function to both human urogastrone and transforming growth factor (TGF α). The addition of EGF to cell cultures induces many cellular responses including morphological changes, stimulation of various active transport systems for both inorganic ions and low molecular metabolites, phosphoinositol turnover and the formation of macromolecules including RNA, protein and DNA (for review see Staros *et al.* 1985). It is detectable in saliva, milk and urine, but in the circulation of man is found primarily in the α granules of blood platelets (Oka & Orth 1983). Its synthesis in mouse salivary glands is induced by both androgens and β-adrenergic agonists (Carpenter *et al.* 1979).

Uterine leiomyomata (fibroids) are benign tumours of the myometrium occurring in up to 25% of women of reproductive age. They may grow to a considerable size and can cause appreciable morbidity since they are a cause of infertility and pregnancy loss as well as commonly leading to menstrual problems. They are oestrogen dependent since they grow during pregnancy, decrease in size after the menopause, and their size can be successfully reduced with LHRH agonists which induce a state of hypo-oestrogenism (Healy *et al.* 1984; West *et al.* 1987). However, it is not known if oestrogen acts

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directly on the uterus or through other mechanisms.

EGF binds to all the cell types within the human uterus, and no changes have been observed during the menstrual cycle (Chegini *et al.* 1986). The number of binding sites is higher in the cervix than in the uterine fundus (Sheets *et al.* 1985). High affinity sites have been demonstrated in women by a number of workers (Sheets *et al.* 1985; Hofmann *et al.* 1984) and in the rat uterus (Mikku & Stancel 1985).

In the rat, the binding of EGF to the uterus is regulated by oestrogen (Mikku & Stancel 1985). In addition, oestradiol also increases the EGF content of mouse uterus (Gonzalez *et al.* 1984). Oestrogen has also been shown to induce the production of EGF-related polypeptides from MCF-7 human breast cancer cells (Dickson *et al.* 1986). It was thus of interest to investigate the influence of oestrogen on the binding of EGF to the human uterus and fibroids which might indicate a role for EGF in fibroid growth.

Patients and methods

The superactive GnRH$_A$, Zoladex [D-ser (t Bu)^6 Azgly^{10}-GnRH-ICI 118630] was the gift of ICI Pharmaceuticals Ltd. Radiolabelled [^{125}I] EGF and unlabelled EGF (isolated from male mouse submaxillary gland) were the gifts of Dr K. Brown, Institute of Animal Physiology, Babraham, Cambridgeshire, UK. All chemicals and reagents were obtained from Sigma Chemical Co. Ltd (Poole, Dorset, UK) or BDH Ltd (Dorset, UK).

Two groups of patients were recruited from those attending the Royal Infirmary, Edinburgh. All were on the waiting list for total abdominal hysterectomy and had uterine fibroids confirmed by ultrasound scan. They were all premenopausal with normal, regular menstrual cycles.

Treatment group

Thirteen women were recruited who after history taking and examination were found to have no contraindication to receiving the GnRH agonist Zoladex. Written informed consent was obtained in each case. The uterine volume was measured by ultrasound, a venous blood sample taken for gonadotrophin and steroid measurement and then the first Zoladex depot administered. All patients received three depots at monthly intervals by subcutaneous injection into the anterior abdominal wall, and had an adominal hysterectomy at a mean 18 (SEM 3) days after the final implant; i.e. treatment was received for about 11 weeks before operation. The start of treatment was not standardized to a particular phase of the menstrual cycle as once suppression is achieved (always within 4 weeks), the endocrinology is the same whether the first depot is given in the follicular or secretory phase (West *et al.* 1987).

Untreated group

Fourteen patients, free from any severe medical disease, were recruited at the time of admission for hysterectomy. This group had received no treatment and were not taking any hormone preparations such as oral contraceptives or progestogens. A full gynaecological history was obtained.

Tissue collection

After the removal of the uterus, its dimensions and volume were measured. Each fibroid was dissected clear of surrounding myometrium or peritoneum and its dimensions measured. Biopsies were taken from each fibroid and from the myometrium adjacent to one of the major fibroids. The tissue was frozen in liquid nitrogen and stored until assayed (usually <1 week). A frozen section was made from each fibroid for histological analysis.

Dating of the menstrual cycle

An endometrial biopsy was taken and placed in formal saline for histological dating (Noyes *et al.* 1950). A peripheral blood sample was taken during the operation for the measurement of gonadotrophins and steroids. The stage of the menstrual cycle was determined from the endometrial histology, the date of the last menstrual period and the plasma progesterone.

Preparation of tissue homogenates

The preparation of the tissue homogenates was based on the method of Hofmann *et al.* (1984). Tissue was removed from liquid nitrogen and 300–500 mg chopped into small pieces. Two millilitres of ice-cold homogenizing buffer (10 mM Tris-HCl, pH 7·0, containing 250 mM sucrose, 1

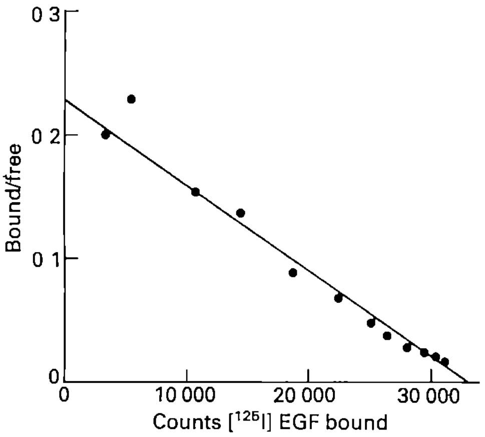

Fig. 1. Scatchard analysis of ^{125}I binding to myometrium. The Kd was calculated from the reciprocal slope.

mM $CaCl_2$ and 0·1% gelatine) were added and the tissue homogenized on ice using a Polytron small blade homogenizer for 5 s at full speed. The volume of homogenate was adjusted to 100 mg/ml and stored at −20°C overnight before assaying.

Measurement of EGF binding

Triplicate samples of 0·2 ml tissue homogenates were incubated in 0·2 ml 0·5% BSA, 10 mM Tris-HCl, pH 7·0, containing approximately 100 000 ct/min radiolabelled EGF. After incubation at room temperature for 2 h, bound hormone was separated (except where indicated) by polyethylene glycol (PEG) precipitation. The separation step was performed on ice to minimize dissociation of bound tracer during separation of bound and free hormone. Briefly, 0·5 ml ice-cold 0·5% bovine γ-globulin (in 40 mM Tris-HCl, pH 7·4) was added to each tube followed immediately by 1 ml ice-cold PEG (8000 mol. wt; 25 g/100 ml 40 mM Tris-HCl, pH 7·4). After vigorous mixing, the tubes were centrifuged at 3200 r/min for 10 min in a refrigerated (4C) MSE Coolspin (Crawley, Sussex, UK) centrifuge. Supernatants were rapidly vacuum aspirated and the radioactivity in the pellets was counted in a NE 1600 (Nuclear Enterprises, Edinburgh, UK) β counter at an efficiency of 75%. Non-specific binding was measured in duplicate in the presence of 200 ng EGF. Specific binding was calculated by subtracting activity bound in the presence of excess unlabelled hormone from total binding in the absence of unlabelled hormone. Background radioactivity (blank) was estimated by incubating and separating buffer and [^{125}I] EGF in the absence of homogenate.

The protein content of the homogenate was measured by the method of Bradford (1976).

Statistics

Non-parametric tests of significance were used.

Results

General characteristics

The patients did not differ significantly in height (treated (T): 162·6 (3·8) cm; untreated (U): 165·1 (2·1) cm), weight (T: 64·9 (5·5) kg; U: 63·0 (6·8) kg), parity (T: 1·8 (0·5); U: 2·0 (0·4) or age (T: 44·3) (1·1) years; U: 44·1 (1·1) years. All results mean (SEM). There was also no significant difference in the uterine volume at presentation (T: 351 (190–1370) ml; U: 400 (200–1080) ml) median (range).

Recovery of bound hormone

The method described by Hofmann *et al.* (1984) for the separation of bound and unbound hormone utilizes centrifugation rather than PEG precipitation. The two methods were compared for four tissues. The recovery of EGF bound to myometrial homogenate was 1·75 (0·5)% of total added counts, mean (SE), following sepa-

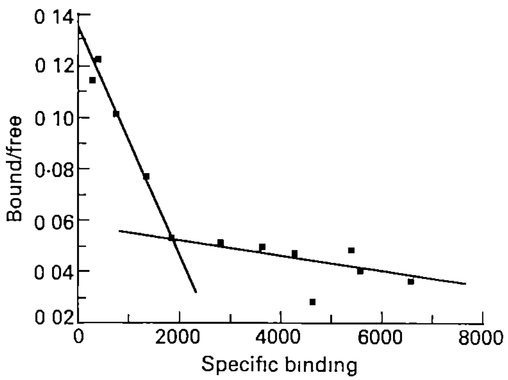

Fig. 2. Scatchard analysis of ^{125}I binding to fibroid The Kd was calculated from the reciprocal slope which with the intercepts was calculated by least square curve fit analysis The correction for low affinity site contribution to high affinity site binding was made and the *x*-intercepts can be used to calculate specific binding capacity.

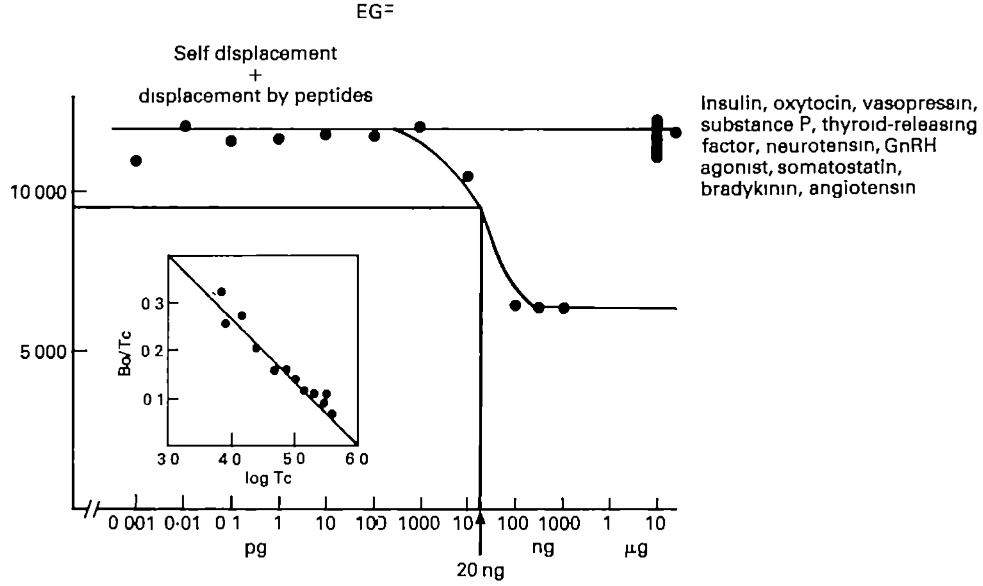

Fig. 3. This illustrates the displacement of [^{125}I] EGF by increasing concentrations of unlabelled EGF. The [^{125}I] EGF was not displaced by the peptides listed in the figure. Bo, Counts bound; Tc, total counts. The graph in the inset is used for the calculation of specific activity.

ration by centrifugation, and 9·2 (0·7)% following PEG precipitation. The latter method was therefore preferred. ^{125}I-labelled EGF was adsorbed to plastic tubes, giving a high background (8–10% of added counts). However, when siliconized glass tubes were used, this value dropped to 2–3% of added counts. All measurements were therefore performed in siliconized glass tubes.

Characterization and properties of EGF binding

Specific binding of ^{125}I-labelled EGF to uterine tissue had a pH optimum of 7·0: non-specific binding increased progressively at acid pH values. The magnitude of binding at 4°C and 25°C was similar and maximum specific binding was reached after 1–3 h. Specific binding at 37°C was significantly less than at 4°C or 25°C (mean % counts bound when incubated at 4°C and 25°C for 2 h = 6·7 (SEM 0·05) compared with 2·9 (SEM 0·02), (n = 4) at 37°C).

Scatchard plots derived from curves of the inhibition of labelled EGF binding by unlabelled EGF gave mean Kd values of 0·56 (SD 0·14) × 10^{9} M^{-1} for three different myometrial homogenates (Fig. 1).

The binding to fibroid tissue appeared to be biphasic (Fig. 2) with dissociation constants of 0·9 (SE 0·05) × 10^{-9}M for the high affinity site and 8·4 (SE 0·7) × 10^{-9}M for the low affinity site, with specific binding capacities of 26 (SE 6·4) fmol/mg protein and 108 (SE 18) fmol/mg protein for low and high affinity sites respectively (n = 4).

Specificity of binding

The binding of EGF to uterine membranes was specific (Fig. 3). Unlabelled EGF decreased the binding of [^{125}I] EGF to membrane preparations, whereas unlabelled insulin, oxytocin, vasopressin, somatostatin, GnRH agonist, thyroid-releasing factor, angiotensin, neurotensin, substance P and bradykinin did not.

EGF binding during the menstrual cycle

Specific binding of EGF to homogenates of fibroid and myometrium in five secretory and seven proliferative uteri are shown in Table 1 and indicate no change during the menstrual

Table 1. Specific binding of EGF to uterine tissue homogenates during the menstrual cycle

	Myometrium (fmol/mg protein)	Fibroid (fmol/mg protein)
Proliferative	13	18
($n = 7$)	(9·2–39)	(8·3–44·2)
Secretory	14	18
($n = 5$)	(6·7–19)	(6·7–31·4)

Results are median (range).

Table 2. Specific binding of EGF to uterine tissue homogenates following the administration of GnRH agonist

	Fibroid (fmol/mg protein)	Myometrium (fmol/mg protein)
Treated	10	19·8
($n = 12$)	(5·5–45·7)	(7·7–47)
Untreated	24	17·5
($n = 12$)	(8·2–44·2)	(6·7–39)

Results are median (ranges).
The difference between the two groups was significant ($P<0·01$; Wilcoxon signed rank test).

cycle. The results of one woman subsequently found to have elevated gonadotrophins were excluded from the above calculation. Results for this woman were: myometrium 39 fmol/mg protein and fibroid 24 fmol/mg protein, which is within the range quoted above for women with normal menstrual cycles.

EGF binding after GnRH agonist treatment

The results from the 12 untreated women and 12 treated women are shown in Table 2. Specific binding of ^{125}I-labelled EGF to fibroid tissue from untreated women was significantly greater than to tissues from women treated with GnRH agonist. Binding to myometrial homogenates remained unchanged.

The correlation of EGF binding with circulating steroid concentrations

Table 3 indicates that in those patients treated with agonist, binding of EGF strongly correlated with circulating oestradiol levels. (The Spearman correlation test was used as EGF binding is not normally distributed.) However, in the untreated group, EGF binding and circulating oestradiol were not correlated.

Discussion

This study confirms that of Hofmann *et al.* (1984) and Sheets *et al.* (1985) which demonstrated specific binding of EGF to human myometrium and fibroids. However, the magnitude of binding achieved in our study was much greater than that reported by Hofmann *et al.* (1984) and agrees with the findings of Sheets *et al.* (1985) in human uterus and Lev-Ran *et al.* (1984) for liver microsomes. The most likely explanation lies with the methods since a different separation step of bound and free was used. In each study Hofmann *et al.* (1984) used low speed centrifugation, Sheets *et al.* (1985) high speed centrifugation, whereas we used polyethylene glycol precipitation. Sheets *et al.* (1985) suggest that centrifugation at this speed (4800 *g*) may underestimate the amount of peptide tracer bound due to low recovery. Since PEG precipitates the hormone-receptor complex, even soluble receptors can be recovered by our method.

There was no change in the specific binding of

Table 3. The relation between plasma oestradiol and the binding of EGF to the human uterus

Variable 1	Variable 2	Significance
(a) Patients treated with GnRH agonist		
Plasma oestradiol	Myometrial EGF-R	0·917 $P<0·01$
	Fibroid EGF-R	0·863 $P<0·01$
	Mean fibroid volume	0·834 $P<0·01$
(b) Patients not treated with GnRH agonist		
Plasma oestradiol	Myometrial EGF-R	0·284 NS
	Fibroid EGF-R	0·268 NS
	Mean fibroid volume	0·407 NS

Statistical analysis used Spearman's correlation test.

EGF to uterine tissues during the menstrual cycle which confirms previous studies (Hofmann *et al.* 1984; Chegini *et al.* 1986). However, when women were rendered hypo-oestrogenic with LHRH agonist, decreased binding of EGF to fibroid but not to myometrium was noted. This indicates that changes in the concentration of oestradiol within the range found in the normal cycle do not affect binding. The mean oestradiol concentration in the treated group was 50 (SE 0·3) pmol/l, whereas in the untreated group it was consistently above this level at 98·6 (SE 25·5) (P<0·01). In the treated group, EGF binding significantly correlated with the circulating oestradiol to fibroid and myometrium level: this was not so for the untreated group. This suggests a 'dose-response' effect of oestradiol at low circulating concentrations which reaches a plateau at levels normally found in premenopausal women. Further studies are needed to investigate this in greater depth.

Uterine fibroids decrease in size when LHRH agonists are administered. This is accompanied by decreased binding of EGF to fibroid tissues and not to myometrium. This differential effect suggests that part of the role of oestrogen in fibroid growth may be mediated by EGF. Altered binding to both tissues would have made a coincidental effect of oestrogen more likely.

Other factors, such as TGFα, bind to the EGF receptor, and this could obviously affect the result of our binding studies. Moreover, oestrogen could affect the binding of other agents to the receptor either as positive or negative regulators or affect receptor affinity or synthesis directly. Oestrogen, therefore, could control fibroid growth by affecting more than one mechanism.

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Association between hormonal and haemorheological changes during the menstrual cycle in healthy women

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Summary. Blood rheology was studied in 15 healthy women throughout a normal menstrual cycle. Significant increases in fibrinogen, blood and plasma viscosity, and a reduction of erythrocyte filtrability were demonstrated during the follicular and ovulatory phase in comparison with mid- and late luteal phase of the menstrual cycle. Positive correlations were found between oestradiol levels and the haemorheological variables. Our data suggest that ovarian hormonal activity influences haemorheology and blood flow in healthy women.

Haemorheological variations have been reported during the normal menstrual cycle and may be related to changes in ovarian hormonal activity (Solerte *et al.* 1985).

At present little is known about the influence of oestrogen and progesterone on the rheology of the blood but the topic is important as oestrogens and oral contraceptive steroids may induce alterations both in blood flow and coagulability. These changes may result in an increased prevalence of cardiovascular problems such as myocardial infarction, thromboembolic disease, hypertension and stroke.

Blood hypercoagulability and the risk of venous thromboembolism have been generally attributed to oestrogenic activity and in particular to the oestrogen content of oral contraceptives (Goodrich & Wood 1966; Astedt *et al.* 1973; Poller *et al.* 1971, 1972).

Haemorheological changes may also have a role in the occurrence of changes in blood flow and thrombosis (Lowe & Forbes 1981; Lowe *et al.* 1980; Buchan & MacDonald 1980).

The present work examines the relation between blood rheology and hormonal pattern throughout the menstrual cycle in healthy women.

Subjects and methods

After giving informed consent, 15 healthy women, mean age 25 years (SD 3·9, range 19–34), with normal body weight (body mass index: weight/height^2 (kg/m^2) within 20–23) were studied during a spontaneous menstrual cycle. None had hypertension or any history of metabolic abnormalities, thrombophlebitis, varicosities or gynaecological disease. None of the women had ever taken any contraceptive pill. They were investigated on day 7 (follicular phase), day 14 (ovulatory phase) and on days 21, 25, and 27 (mid- and late luteal phases).

After an overnight fast, blood samples were taken without a tourniquet in the sitting position between 08.00 and 09.00 hours. The samples were anticoagulated with EDTA-2 Na for estimation of haematocrit, FSH, LH, oestradiol, progesterone, erythrocyte filtrability, whole blood and plasma viscosity. Blood was anticoagulated with 0·129 M trisodium citrate (9:1 v/v) to obtain plasma for measurement of fibrinogen. FSH and LH were determined by radioimmunoassay (Sorin Biomedica, Saluggia,

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Italy); the detection limits were 0·15 U/l and 0·25 U/l respectively, and the intra- and interassay variations were within 1% and 7%. Oestradiol was measured by radioimmunoassay (Sorin Biomedica, Saluggia, Italy); the minimum detectable dose was 12 pmol/l and the intra- and interassay variations were 5% and 8% respectively. Progesterone was determined by radioimmunoassay (DPC, Los Angeles, CA, USA); the detection limit was 0·15 nmol/l and the intra- and interassay variations were 5% and 7% respectively. The oestradiol/progesterone ratio (E_2/P) was calculated. Blood glucose, triglycerides, total and HDL-cholesterol were estimated by enzymatic procedures. Plasma fibrinogen was determined by immunoelectrophoresis (Laurell 1966) by using a specific antiserum (Clotimmun Fibrinogen, Behring Institute, Scoppito, Italy). Plasma apolipoprotein levels (A, A_1 and B) were estimated by immunoelectrophoresis (Laurell 1966) using specific antisera (Behring Institute, Scoppito, Italy). Blood, plasma and serum viscosity were measured at 37°C with a Brookfield digital cone/plate viscometer (Brookfield, Inc., Stoughton, MA, USA); measurements were done at shear rates of 450, 90, 4·5, 2·25 s^{-1} with a 0·8° cone spindle; the coefficient of variation of the method was below 3%. Results were expressed in mPa.s. Erythrocyte filtrability was measured at 37°C as whole blood filtrability by the method of Reid *et al.* (1976); blood was filtered under a negative pressure of 20 cm water, through 5 μm (ϕ) polycarbonate filters (Hemafil, Nuclepore, Pleasanton, CA, USA).

The statistical analysis was carried out by conventional methods: Wilcoxon's test for paired comparisons, and Pearson's correlation coefficient. Results are given as mean and standard deviation (SD).

Results

Table 1 shows the variations in the hormonal and biochemical variables during the menstrual cycle. No changes in blood glucose, triglyceride, total or HDL-cholesterol or apolipoprotein levels were found in the study.

Table 2 summarizes the variations in plasma fibrinogen and the haemorheological variables during the menstrual cycle. No changes were found in the haemorheological variables or the fibrinogen levels between day 7 and day 14. In contrast, blood, plasma and serum viscosity and plasma fibrinogen concentrations were higher on days 7 and 14 than on days 21, 25 and 27. The venous haematocrit remained unchanged throughout the study. Significant positive correlations between E_2/P ratio and plasma fibrinogen and between E_2/P ratio and plasma viscosity were demonstrated on day 21 ($r = 0·52$, $P<0·05$; $r = 0·58$, $P<0·05$ respectively) and on day 25 ($r = 0·54$, $P<0·05$; $r = 0·57$, $P<0·05$ respectively), whereas a negative correlation between E_2/P ratio and erthyrocyte filtrability was found on day 21 ($r = 0·66$, $P<0·01$) and on day 25 ($r = 0·62$, $P<0·05$). Significant positive

Table 1. Clinical, hormonal and metabolic variables of the 15 healthy women studied throughout the menstrual cycle

	Day of cycle				
	7	14	21	25	27
Body mass index (kg/m²)	21·5 (1·1)	21·4 (1·1)	21·6 (1·0)	21·3 (1·0)	21·4 (1·1)
FSH (U/l)	7·53 (1·9)	15·4 (3·6)	8·55 (2·3)	7·21 (1·8)	4·71 (1·4)
LH (U/l)	11·4 (5·2)	36·7 (7·6)	12·3 (4·2)	8·71 (4·4)	6·78 (2·9)
Oestradiol-17 (pmol/l)	239 (91)	876 (211)	462 (141)	395 (119)	222 (80)
Progesterone (nmol/l)	2·23 (0·7)	2·70 (1·1)	24·5 (3·7)	25·3 (4·5)	18·8 (3·5)
Blood glucose (mmol/l)	3·96 (0·4)	3·99 (0·4)	4·01 (0·4)	3·88 (0·4)	3·90 (0·4)
Triglycerides (mmol/l)	1·07 (0·3)	1·11 (0·3)	1·05 (0·3)	1·17 (0·3)	1·15 (0·3)
Total cholesterol (mmol/l)	4·69 (0·5)	4·70 (0·5)	4·67 (0·4)	4·75 (0·5)	4·78 (0·5)
HDL-cholesterol (mmol/l)	1·43 (0·1)	1·40 (0·1)	1·46 (0·1)	1·42 (0·1)	1·40 (0·1)
Apolipoproteins (mg/dl)					
A	212 (24)	211 (22)	217 (21)	216 (25)	220 (24)
A1	149 (19)	151 (21)	147 (19)	143 (20)	148 (20)
B	108 (16)	105 (17)	111 (17)	106 (16)	106 (15)

Results are means (SD).

Table 2. Plasma fibrinogen and haemorheological variables in the 15 healthy women studied throughout the menstrual cycle

	Day of cycle				
	7	14	21	25	27
Fibrinogen (mg/dl)	371 (57)*	363 (60)*	321 (39)	311 (37)	330 (41)
Haematocrit (%)	40·2 (0·6)	40·2 (0·6)	40·3 (0·7)	40·4 (0·6)	40·3 (0·5)
Blood viscosity					
SR 450 s^{-1} (mPa.s)	3·34 (0·1)*	3·33 (0·1)*	3·04 (0·1)	3·02 (0·1)	3·00 (0·1)
SR 90 s^{-1} (mPas.s)	5·16 (0·2)*	5·17 (0·2)*	4·74 (0·1)	4·70 (0·1)	4·68 (0·1)
SR 4·5 s^{-1} (mPa s)	8·34 (0·2)*	8·36 (0·2)*	8·05 (0·1)	8·00 (0·1)	8·02 (0·1)
SR 2·25 s^{-1} (mPa.s)	12·8 (1·7)*	12·9 (1·6)*	11·8 (1·5)	11·5 (1·5)	11·6 (1·6)
Plasma viscosity					
SR 450 s^{-1} (mPa.s)	1·51 (0·1)*	1·51 (0·1)*	1·42 (0·03)	1·41 (0·03)	1·43 (0·04)
Serum viscosity					
SR 450 s^{-1} (mPa.s)	1·41 (0·1)*	1·40 (0·1)*	1·32 (0·03)	1·31 (0·01)	1·31 (0·02)
Erythrocyte filtrability (ml/min)	2·98 (0·2)*	3·00 (0·2)*	3·32 (0·2)	3·33 (0·2)	3·27 (0·15)

Results are means (SD).
SR, Shear rate.
* $P<0·01$ compared with days 21, 25 and 27.

correlations between oestradiol, blood viscosity ($r = 0·54$, $P<0·05$ at SR 4·5 s^{-1}; $r = 0·66$, $P<0·01$ at SR 2·25 s^{-1}), fibrinogen ($r = 0·58$, $P<0·05$) and plasma viscosity ($r = 0·54$, $P<0·05$) were observed on day 14 concomitantly with a negative correlation between oestradiol and erythrocyte filtrability ($r = 0·68$, $P<0·01$).

Discussion

As in our previous study (Solerte *et al.* 1985) several changes in blood rheology were found in healthy women during the menstrual cycle.

A number of factors, such as posture, fasting, circadian rhythm, stress, and the method used to obtain the blood sample, can affect the results of haemorheological measurements. Consequently, our study was performed under identical conditions for each subject examined. As a result the haemorheological changes observed during the study should be strictly dependent on the hormonal variations related to ovarian activity during the menstrual cycle.

Increased blood viscosity (both at high and low shear rates), plasma and serum viscosity, and reduced erythrocyte filtrability, were demonstrated during the follicular and ovulatory phase of the menstrual cycle. These changes might be related to the oestrogenic activity normally occurring during this phase of the cycle, and we found several correlations between plasma concentrations of oestradiol, the increase of blood and plasma viscosity, and the reduction of erythrocyte filtrability on day 14 of the cycle, when oestrogen levels were highest. Moreover, oestradiol seems to influence plasma fibrinogen levels which were increased significantly during the follicular and ovulatory phase of the cycle.

The increased plasma fibrinogen concentrations may be responsible for blood and plasma hyperviscosity and for the reduced erythrocyte filtrability observed on days 7 and 14.

In addition to plasma viscosity, serum viscosity was higher in the follicular and ovulatory phases than in mid- and late luteal phases of the menstrual cycle. Other proteins, such as acute-phase proteins, may therefore be influenced by oestrogens and may be responsible for the rheological changes.

On the other hand, the lower viscosity pattern found in the luteal phase of the menstrual cycle may be dependent on progesterone levels. In the luteal phase of the cycle there were several significant correlations between the E_2/P ratio, plasma viscosity, plasma fibrinogen and erythrocyte filtrability. Oestrogenic activity seems to increase blood viscosity whereas progesterone seems to decrease it.

Blood lipid variations can influence blood rheology. Blood viscosity is increased and erythrocyte deformability reduced in primary hyperlipoproteinaemia (Leonhardt & Arntz

1978) and in diabetic hyperlipidaemia (Patti *et al.* 1986). However, in our study we found no changes in serum triglycerides, total and HDL-cholesterol or plasma apolipoprotein levels during the menstrual cycle.

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Oligohydramnios and fetal cerebral blood flow

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Summary. Blood flow velocity waveforms in the fetal internal carotid artery were recorded in five pregnancies complicated by prolonged severe oligohydramnios due to bilateral renal agenesis. End-diastolic flow velocity was reduced, absent or even reversed, resulting in a raised pulsatility index in all five cases. These data suggest that prolonged severe oligohydramnios may hamper cerebral blood flow through fetal head compression.

Recently, Wladimiroff *et al.* (1986) introduced a pulsed Doppler method for recording the flow velocity waveform in the fetal internal carotid artery. It was demonstrated that in the human fetus a brain-sparing effect may occur in the presence of intrauterine growth retardation (IUGR) due to uteroplacental insufficiency (Wladimiroff *et al.* 1987; Arbeille *et al.* 1987). However, in growth-retarded fetuses associated with structural or chromosomal defects, normal flow velocity waveforms in the internal carotid artery were observed (Wladimiroff *et al.* 1987). Prolonged severe oligohydramnios may particularly be encountered in the presence of gross structural anomalies such as bilateral renal agenesis.

The object of the present study was to assess the possible effect of cranial compression on cerebral blood flow in the presence of prolonged severe oligohydramnios.

Patients and methods

Five pregnancies with prolonged severe oligohydramnios were referred for assessment of fetal anatomy and growth at between 26 and 29 weeks gestation. All five fetuses presented in the breech position. Although the presence of marked oligohydramnios restricted the quality of fetal imaging, a diagnosis of bilateral renal agenesis could be made in all five patients. Flow velocity waveforms from the fetal internal carotid artery were obtained using a Diasonics CV 400 combined mechanical sector and pulsed Doppler system (carrier frequency 3·5 and 3

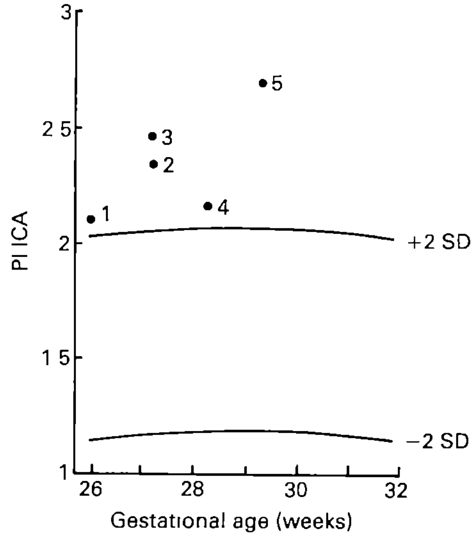

Fig. 1. Pulsatility index (PI) in the internal carotid artery (ICA) of five fetuses with bilateral renal agenesis shown against the normal values established by Wladimiroff *et al* (1988).

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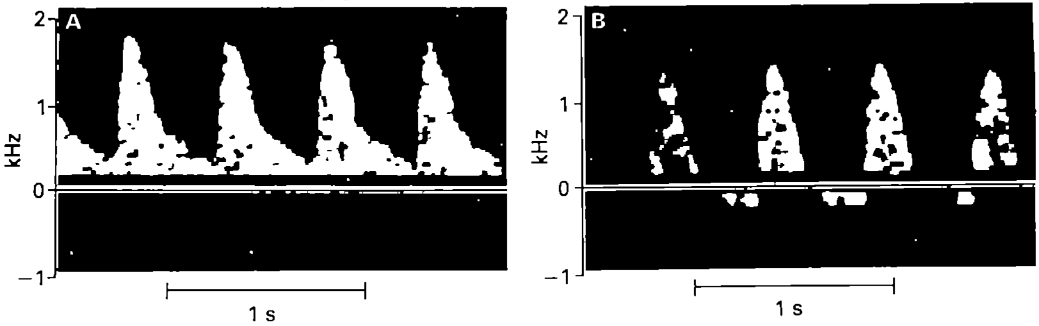

Fig. 2. (A) Normal flow velocity profile in the fetal internal carotid artery at 28 weeks gestation. Note the presence of forward flow throughout the cardiac cycle (B) Flow velocity profile in the fetal internal carotid artery depicting reversal of end-diastolic flow (case 5)

MHz) (Wladimiroff *et al.* 1986). The degree of pulsatility of the waveforms was quantified by calculating the pulsatility index (PI) according to Gosling & King (1975). The PI merely reflects peripheral vascular resistance (Noordam *et al.* 1987). PI data were plotted against the normal range established by Wladimiroff *et al.* (1988).

There were two intrauterine and three early neonatal deaths. In three pregnancies fetal birthweight was below the 5th centile for gestational age according to the tables of Kloosterman (1970). Autopsy confirmed the diagnosis of bilateral renal agenesis in all five patients.

Results

Figure 1 demonstrates that the PI values in the fetal internal carotid artery from all five fetuses are above the upper limit (2 SD) of the normal range. End-diastolic flow velocity in the internal carotid artery was reduced in one fetus (case 1), absent in three fetuses (cases 2–4) and even reversed in the remaining fetus (case 5; Fig. 2).

Discussion

The raised PI in the fetal internal carotid artery in the presence of severe oligohydramnios suggests increased resistance to cerebral blood flow. A similar rise in PI was demonstrated in the presence of hydrocephaly (van den Wijngaard *et al.* 1988*a*), and was attributed to stretching, displacement, deformation or even compression of the fetal internal carotid arteries by the enlarged ventricles.

The well-known non-renal features of renal agenesis are frequently considered the result of compression of the fetus due to the severe oligohydramnios (Thomas & Smith 1974). Recently, we described a case of umbilical cord compression under similar conditions, in which a high resistance flow velocity waveform in the umbilical artery became normal following amnioinfusion of 150 ml of a 5% glucose solution (van den Wijngaard *et al.* 1988*b*). In the present study, all five fetuses demonstrated a characteristic dolichocephalic skull shape, a deformation thought to be a reflection of intrauterine environmental factors, such as oligohydramnios (Kasby & Poll 1982), breech position (Sunderland 1981) or twin pregnancy (Socol *et al.* 1984). A common causal mechanism is likely to be cranial compression by the uterine wall (Houlton & Brennan 1976). Doppler studies in the new-born have clearly demonstrated that moulding of the skull compresses the superior sagittal sinus resulting in decreased cerebral blood flow (Newton & Gooding 1975). We postulate a similar mechanism for the observed elevation of fetal cerebral vascular resistance in the presence of prolonged severe oligohydramnios.

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The management of multiple pregnancy in women with a lower-segment caesarean scar. Is a repeat caesarean section really the 'safe' option?

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Summary. Multiple pregnancy occurring in a patient who has previously had a lower-segment caesarean section is an unusual sequence of events. Retrospective analysis over an 11-year period in Sheffield from 1975 to 1985, revealed 25 cases, a rate of 1 in 3300 deliveries. The caesarean section rate in this group of women rose from 20% (3/15) in the first six years to 70% (7/10) in the latter years for no obvious reason. This change in management did not produce any improvement in fetal outcome but there was an increase in maternal morbidity. This analysis and a review of available literature suggest that multiple pregnancy is not in itself an indication for elective repeat caesarean section.

Although previous caesarean section alone remains an indication for elective repeat caesarean section in the USA, British opinion generally favours a trial of labour when the caesarean section was performed for a non-recurring cause and a new indication has not developed in the current pregnancy. Such a policy is associated with lower maternal mortality and morbidity with no increase in perinatal mortality or morbidity (O'Sullivan *et al.* 1981; Boucher *et al.* 1984).

Case *et al.* (1971) cited multiple pregnancy as a contraindication to trial of scar arguing that overdistension of the uterus would weaken the scar and although no evidence was produced to support this contention, this concept has found its way into some undergraduate textbooks (Clayton *et al.* 1985). The large body of literature which now exists attesting to the safety of trial of scar in general either omits to mention twin pregnancy or cites it as a contraindication.

We have identified seven papers which specifically mention 30 twin pregnancies allowed a trial of scar and there is no record of maternal or perinatal mortality or scar dehiscence (Table 1).

One paper referred to a set of twins electively delivered by a repeat caesarean section (Birnbaum 1956).

We have performed a retrospective analysis of all twin deliveries in Sheffield in women with a lower uterine segment scar over the 11-year period from 1975 to 1985 to assess management of the delivery and record the outcome.

Patients and methods

The study covered the years 1975–1985 inclusive for all three maternity hospitals serving the City of Sheffield (Nether Edge Hospital, Northern General Hospital, and the Jessop Hospital for Women).

The relevant background information relating to all multiple pregnancies, caesarean sections, and perinatal deaths, in these hospitals during the years studied was obtained from the Trent Regional Health Authority's data storage system. The records of all patients with a history of multiple pregnancy and previous lower-segment caesarean section were identified. This

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Table 1. Management of multiple pregnancy in women with uterine scars—review of literature

Mode of delivery	Authors	No of pregnancies
Vaginal ($n = 30$)	Schmitz & Gajewski (1951)	5
	Pedowitz & Schwartz (1957)	3
	Allahbadia (1963)	3
	Browne & McGrath (1965)	13
	O'Connell (1966)	3
	Meehan *et al.* (1972)	1
	Wadhawan & Narone (1983)	2
Repeat caesarean section ($n = 1$)	Birnbaum (1956)	1

information was double-checked against the birth statistics in the individual hospitals.

Statistics

The statistical significance of the observed associations was assessed using Pearson's χ^2-test with Yates' correction for the 2 × 2 tables.

Results

Of the 79 129 total deliveries in Sheffield during the 11 years studied 810 were multiple births, an overall rate of about 1%. The incidence of multiple births altered very little during the individual years examined (Fig. 1), but the proportion of women with multiple pregnancies delivered by caesarean section fluctuated quite markedly from 10% in 1975 to 31% in 1985. In the 6 years between 1975 and 1980 inclusive, 15% of the 427 multiple pregnancies were delivered by caesarean section, compared with 22% of the 383 multiple pregnancies between 1981 and 1985, and this increase in the caesarean section rate for multiple births was statistically significant (χ^2 6·0; d.f. = 1, $P = 0{\cdot}01$). This increase in the caesarean section rate for multiple pregnancies could not be explained by an increase in the caesarean section rate for all pregnancies as this remained around 11% (Table 2). Furthermore, this increase in the use of caesarean section did not appear to have a beneficial effect on the perinatal mortality for multiple pregnancies. The uncorrected perinatal mortality for the 166 babies born by caesarean section between 1980 and 1985 was 30/1000 compared with 22/1000 for the 133 babies born between 1975 and 1980. The uncorrected perinatal mortality for all births steadily improved during these years, as did that for multiple births in general and multiple births born vaginally (Fig. 2).

There were 25 instances of multiple pregnancy in women with a lower-segment scar (3/10 000 of all births and 3% of multiple births). Twenty-four of these were twin pregnancies and there was one triplet pregnancy. There was a marked increase in the caesarean section rate in this small group of women from 20% (3/15) in the first part of the study period, 1975–1980, to 70% (7/10) between 1981 and 1985. Although the numbers involved are small this difference was statistically significant ($\chi^2 = 4{\cdot}34$; d.f. = 1, $P = 0{\cdot}037$). Nine of the 10 caesarean sections were elective. None of the women planned for vaginal delivery required a caesarean section in labour. Twenty-three of the 25 pregnancies were delivered after 35 completed weeks. Of the two pregnancies terminating before 35 weeks, one was a triplet pregnancy which ended at 25 weeks with spontaneous labour and vaginal delivery and the other was a twin pregnancy delivered by an emergency caesarean section at 28 weeks for severe hypertension.

In five of the seven patients delivered by caesarean section between 1981 and 1985 the primary or sole indication for the section appeared to be the combination of the twin preg-

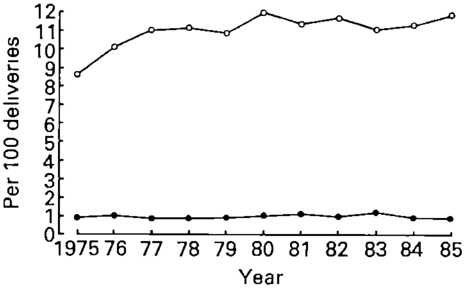

Fig. 1. Incidence of multiple pregnancies (●) and caesarean section rate (○) for all pregnancies in the City of Sheffield 1975–85.

Table 2. Relation between caesarean sections for multiple births and caesarean sections for all births in the city of Sheffield (1975–1985)

	Mode of delivery	1975–1980 *n* (%)	1981–1985 *n* (%)
All births	Caesarean section	4612 (11)	4051 (11)
	Vaginal delivery	38 894 (89)	31 572 (89)
Multiple births	Caesarean section	65 (15)	85 (22)
	Vaginal delivery	362 (85)	298 (78)

nancy and uterine scar. No patient had a caesarean section for this indication between 1975 and 1980. Over this period the essential characteristics of the patients and their pregnancies did not seem to have altered. In 13 of the 15 patients (87%) delivered between 1975 and 1980, and eight of the 10 patients (80%) delivered between 1981 and 1985, the first twin was a vertex presentation.

Pregnancy outcome

Vaginal deliveries. The 15 patients who were allowed to labour achieved successful vaginal deliveries. There was no clinical evidence of scar rupture in any of the patients. In 12 patients the scar was checked manually and found to be intact. The mean duration of labour was 6·8 h (SD 3·5, range 3–19). Oxytocin was used in five patients (to induce or augment labour) and epidural analgesia was used in 13 of the 15 patients. Of the 51 babies 39% were born by forceps or the ventouse, 16% were assisted breech deliveries. In one instance the second twin was born by breech extraction and in another instance internal podalic version and breech extraction was used to deliver the second twin. Of the 15 patients delivered vaginally three had recorded blood losses between 500 and 800 ml. The postnatal period in all 15 was uneventful.

Caesarean section. In one patient a 3-cm window was observed in the lower segment during the elective repeat lower-segment caesarean section. One patient developed postoperative large bowel obstruction and a second laparotomy was required to drain a pelvic abscess. The postoperative period was further complicated by a chest infection. Two other patients had experienced pyrexial morbidity due to wound infections.

Fetal outcome

The perinatal mortality rate in this group of babies was high at 98/1000. The perinatal loss was entirely due to early neonatal deaths (5/51) and was related to congenital abnormality and prematurity. None of the deaths was attributable to the mode of delivery (Table 3).

Discussion

Perinatal mortality is substantially higher in multiple pregnancies than in singleton pregnancies but the evidence suggests that much of this observed increase in perinatal loss relates to the complications of preterm delivery rather than to intrapartum problems (McCarthy *et al.* 1981). No improvement in fetal outcome has been obtained through the more liberal use of caesarean section in twin pregnancy (Medearis *et al.* 1979; Chervenak *et al.* 1984; Bell *et al.* 1986) and therefore from the available evidence multiple pregnancy alone cannot be seen as an indication for caesarean section. It is interesting, however, that there appears to be an increase in the use of caesarean delivery in twin pregnancies since 1970 in our own population, other UK cities

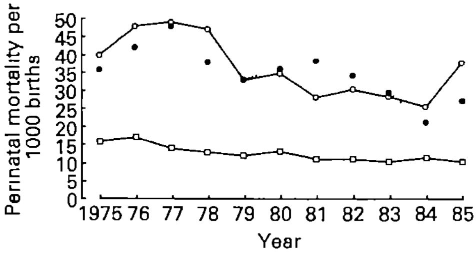

Fig. 2. Perinatal mortality figures for singleton and multiple births in the City of Sheffield 1975–85 ○, Perinatal mortality for multiple births delivered vaginally (uncorrected); ●, perinatal mortality for multiple births (uncorrected); □, perinatal mortality for all births (uncorrected).

Table 3. Adverse fetal outcome and mode of delivery in 25 multiple pregnancies in women with uterine scars

	Mode of delivery	1975–1980 ($n = 30$)	1981–1985 ($n = 21$)	Total
1-min Apgar score ≤4	Caesarean section	0	2†‡	2
	Vaginal delivery	0	3§	3
Neonatal deaths	Caesarean section	0	1‡	1
	Vaginal delivery	1*	3§	4

* One of twins born vaginally at 36 weeks. Weight 1600 g. Apgar scores 5 and 7 at 1 and 5 min. Cause of death multiple congenital abnormalities.

† One of twins born as breech by emergency lower-segment caesarean section at 37 weeks. Weight 2060 g Apgar scores 4 and 9 at 1 and 5 min. Survived with no apparent ill effects.

‡ One of twins born as breech by emergency caesarean section at 28 weeks. Weight 1900 g. Apgar scores 2 and 7 at 1 and 5 min. Cause of death respiratory distress syndrome.

§ Triplets born spontaneously at 25 weeks. Weight 1060, 780 and 640 g. Died within 36 h due to extreme preterm birth.

(Constantine & Redman 1987) and in the USA (Bell *et al.* 1986).

Does the combination of multiple pregnancy and a lower uterine segment scar constitute an indication for a repeat operation? Does uterine overdistension increase the risk of scar rupture? By reviewing our own records and reviewing the literature 56 cases of multiple pregnancy in women with uterine scars were available for analysis. The only maternal death was in the elective repeat caesarean section group and was due to pulmonary embolism (Birnbaum 1956). Significant morbidity too occurred only in the elective caesarean section group (our series). Only one woman was found to have a scar dehiscence and this was found at elective caesarean section, giving an overall incidence of scar dehiscence of 1·7%. This compares favourably with the incidence of scar dehiscence of 2·9% in singleton pregnancies in Sheffield subjected to a trial of scar, and the quoted figures for other obstetric populations, 0–10% (Meehan *et al.* 1972; Pedowitz & Schwartz 1957; Boucher *et al.* 1984).

None of the five women in our series whose twin pregnancies were complicated by 'marked polyhydramnios' diagnosed clinically and/or ultrasonographically had scar dehiscence and three of these were delivered vaginally. Pedowitz & Schwartz (1957) were unable to find any correlation between increasing fetal weight, polyhydramnios, multiple pregnancy and scar dehsicence. The idea of scar dehiscence due to overdistension, though often suggested, has not been substantiated.

Delivery of the second twin may present problems. Internal podalic version might put the scar at maximum risk, but in the one woman in our series this did not happen.

We conclude from the review of available literature and the results of this study that it does not appear that the combination of twin pregnancy and a lower-segment scar in itself constitutes an indication for repeat caesarean section. Rather, the same strict criteria used to govern the safe conduct of a trial of scar in a singleton pregnancy should be applied as long as the first twin is presenting by the vertex. In the absence of other contraindications a trial of vaginal delivery should not be withheld. The numbers of women requiring this option in a given year will be small in relation to the general obstetric population, hence this policy will have little impact on the overall caesarean section rate. Yet to the individual woman faced with the care of two newborn babies, delivery by a caesarean section with its attendant discomfort, increased hospital stay, and possible morbidity is not necessarily the best option.

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Ultrasound in-utero diagnosis of choroid plexus haemorrhage

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Case report

A 23-year-old woman, gravida 2, para 1, was first seen at 11 weeks gestation when her dates were confirmed by ultrasound. In her previous pregnancy labour had been induced at 38 weeks gestation because of pregnancy-induced hypertension and she was delivered of a normal infant weighing 2·58 kg. At her booking visit in this pregnancy her blood pressure was normal and the pregnancy progressed uneventfully until 33 weeks gestation when she was found to be hypertensive, 140/92 mmHg, with 1+ protein in the urine. Her blood pressure settled on admission to the day care unit and she was sent home to be followed up by the community midwives. At 35 weeks gestation her blood pressure was again elevated to 130/95 mmHg but with no proteinuria and she complained of feeling generally unwell with vomiting and diarrhoea. An initial cardiotocograph was unreactive and biophysical profile showed fetal apnoea but with other features normal. She was therefore admitted to hospital, and repeat cardiotocography over 2 h showed normal variability and no decelerations, but still with no accelerations. As no clinical problem was identified, ultrasound was performed the following day to exclude fetal anomaly. This showed an appropriately grown fetus. The biparietal diameter (BPD) was 88 mm, head circumference 32·4 cm, abdominal circumference 31·8 cm and head to abdominal circumference ratio 1·02. These measurements lie close to the mean for a gestation of 35 weeks (Campbell & Thoms 1977; BPD figures from Campbell & Newman 1971 modified to ultrasound velocity of 1540). The choroid plexus of the right lateral ventricle was noted to be enlarged though ventricular size was normal (Fig. 1a) and the diagnosis of intraventricular haemorrhage was suggested. The fetal anatomy was otherwise normal. That day the patient's general condition had improved, her blood pressure had settled almost immediately after admission, and cardiotocography was reactive. Biophysical profile was 10/10 and the umbilical artery flow velocity waveform was normal. She was discharged home with regular outpatient fetal monitoring which remained normal. At 36 weeks gestation, 10 days after the first abnormal scan, the ultrasound was repeated. On this occasion there was moderate symmetrical dilatation of the lateral ventricles, the third and fourth ventricles were also dilated and the right choroid plexus again appeared enlarged with an irregular outline (Fig. 1b). As there was no evidence that the intrauterine environment was hostile and as no immediate neonatal intervention was felt to be required it was decided after discussion between ourselves and the couple to manage the pregnancy expectantly. However, a repeat ultrasound 4 days later showed persisting hydrocephalus; abdominal growth had been appropriate and the abdominal circumference was 33·2 cm, but head growth appeared excessive, the BPD measured 96 mm, which was 2 SD above the mean for gestation, the head circumference at 35 cm was above the mean and there had been an increase in the head to abdominal

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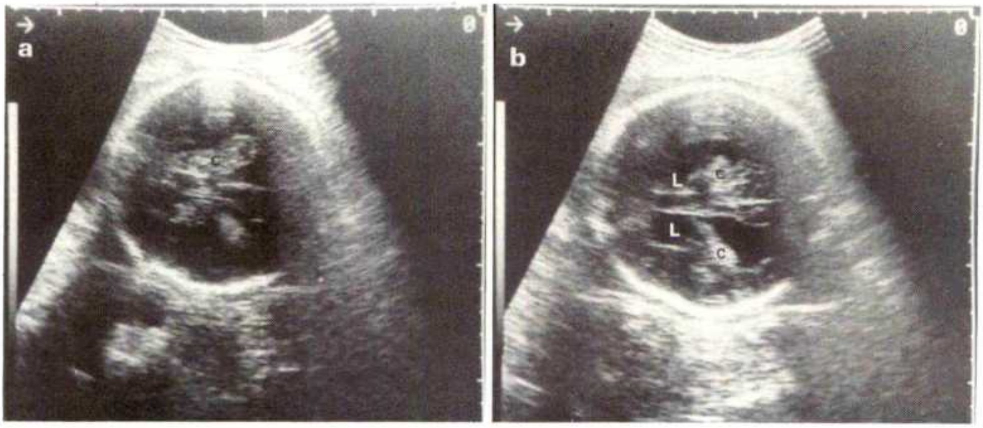

Fig. 1. (a) Transverse section of the fetal head at the level of the bodies of the lateral ventricles. The right choroid plexus, uppermost (c) is enlarged, the ventricles are not dilated. (b) Transverse section at the same level obtained 10 days later. The lateral ventricles (L) are now dilated, the right choroid plexus, uppermost (c) remains enlarged and has an irregular outline. The left choroid plexus (c) is normal.

circumference ratio which was now 1·50. It was therefore decided to perform a caesarean section to avoid the risk of intrapartum damage to the already compromised fetal head.

A male infant weighing 2·7 kg was born with Apgar scores of 8 at 1 min and 9 at 5 min, no resuscitation being necessary. On examination he was alert and active. The head circumference was 35·5 cm (97th centile for gestational age), the anterior fontanelle was full and the sagittal sutures were splayed. The child was irritable with legs extended and showing spontaneous splaying and dorsiflexion of the toes. Examination was otherwise unremarkable. An ultrasound scan confirmed the antenatal findings of hydrocephalus with blood clot attached to the right choroid plexus (Fig. 2a & b); there was no evidence of germinal matrix haemorrhage. On the day of birth, ventricular pressure was elevated at 15 mmHg and Doppler ultrasound studies showed improved blood flow after removal of 15 ml of blood-stained fluid. On the following day a Rickham reservoir was inserted to facilitate repeated pressure studies and the daily removal of accumulating fluid. On the eighth day of life, a right-sided Pudenz-Schultz ventriculoperitoneal shunt was inserted and the infant was discharged home the following week. One month later the child developed a ventriculitis, the shunt was removed and has not needed to be replaced. At the age of 5 months he is now thriving and has a normal clinical examination for age.

Viral cultures were negative in both the mother and infant, and platelet counts were also normal. No cause was found for the mother's diarrhoea and vomiting.

Discussion

We are aware of 14 reports in the literature of the diagnosis of intracranial haemorrhage *in utero*. In six the diagnosis was made after birth, and in eight the diagnosis was made antenatally by ultrasound.

The features of the intracranial bleed described in nine of these reports are not typical of the periventricular–intraventricular haemorrhage (PVH/IVH) seen in premature infants. Lam & Shulman (1985) and Zalneraitis & Young (1979) described five cases in which the bleeding was secondary to iso-immune thrombocytopenia. A further two (Parkinson 1964; Portman & Brouilette 1982) were related to external trauma and amniocentesis respectively. The remaining five reports (Donn *et al.* 1984; Bondurant *et al.* 1984; Mintz *et al.* 1985; Greene *et al.* 1985; Bose 1978) described massive bleeding which was predominantly subarachnoid or intraparenchymal. These five all resulted in intrauterine death.

In five of the reports (Kim & Elyaderani 1982; McGahan *et al.* 1984; Pretorius *et al.* 1986; Lustig-Gillman *et al.* 1983; Jackson & Blumhagen 1983) the features described are in keeping with the changes seen in neonatal PVH/IVH. In four of them the diagnosis was made only after the development of hydrocephalus, one was recognized only after intrauterine death had occurred and one was diagnosed

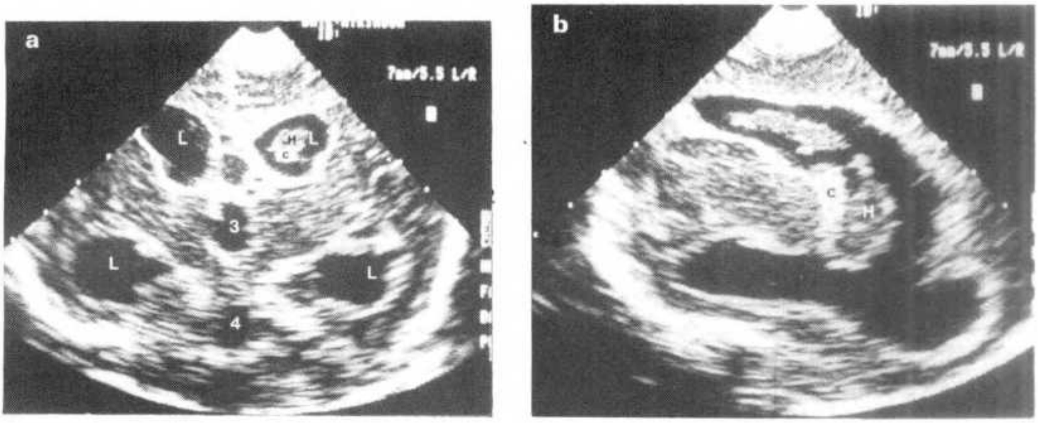

Fig. 2. (a) Coronal section of the neonatal head. There is dilatation of the lateral (L), 3rd (3) and 4th (4) ventricles. Haematoma (H) is seen in the right lateral ventricle adherent to the choroid plexus (c). (b) Sagittal view of the right lateral ventricle in the newborn infant. The ventricle is dilated and there is haematoma (H) attached to the choroid plexus (c).

postnatally. One case (Pretorius *et al.* 1986) was very similar to ours, the initial subependymal bleed was recognized and the development of hydrocephalus indentified in subsequent scans. Outcome was better in this group; three of the infants survived with degrees of handicap ranging from none to mild quadriparesis and developmental delay. In one case there was severe recurrent maternal pancreatitis, in another the birthweight of the infant was below the 5th centile, but in the remaining cases there was no obvious pregnancy complication.

Neonatal PVH/IVH is common, affecting approximately 30% of infants born before 34 weeks gestation and 50% born before 30 weeks gestation (Levene *et al.* 1985). The prevalence of intrauterine PVH/IVH is unknown but it is likely to be an uncommon event; there are few ultrasound reports of the diagnosis despite widespread use of ultrasound in many centres, and where hydrocephalus develops this would be recognized at birth (the rate of isolated neonatal hydrocephalus is only 0·39–0·89 per 1000 total births (Chervenack *et al.* 1983). The frequency of IVH in stillborn infants has been quoted as 6% (Harke *et al.* 1972; Leech & Kohnen 1974). But mild cases of in-utero IVH/PVH could occur without being noted clinically or leading to neonatal problems attributed to intrapartum events. This will become clearer with greater experience of detailed ultrasound in the third trimester. Recently, attention has been focused on the timing of brain damage (Symonds 1987). In-utero PVH/IVH provides clear evidence of intracranial events which may result in cerebral injury.

Neonatal PVH/IVH is seen most often in infants born at less than 32 weeks gestation, and choroid plexus haemorrhage is a lesion that tends to occur in more mature infants. In our patient the haemorrhage was diagnosed at 35 weeks gestation and appeared to originate in the choroid plexus; it is, however, difficult to distinguish between the two types of haemorrhage even in the newborn (Reeder *et al.* 1982) and we cannot be certain of the site of origin of the bleed. The mechanisms for in-utero haemorrhage may be the same as those operating in the newborn, but it is interesting that in our patient there was no clear precipitating event. Term infants with intraventricular haemorrhage, most of whom have choroid plexus bleeds like our infant, have no definable pathogenic factors in approximately 25% of cases (Volpe 1987).

In our patient the diagnosis was fortuitous but it did allow careful fetal monitoring and selection of what was considered to be the optimum time of delivery.

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Treatment of an interstitial pregnancy with actinomycin D.
Case report

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Case report

A woman aged 31 years (gravida 4, para 2) arrived at our clinic on 25 November 1986 with amenorrhea of 6–7 weeks duration and vaginal spotting for the previous week. The uterus was slightly enlarged, with fullness and tenderness in the region of the right tube and ovary. A curettage was performed on the day of admission and the endometrium obtained showed early proliferative changes. Serum human chorionic gonadotrophin (β-hCG) level was 36 m-i.u./ml on 30 October 1986. The patient was discharged with the presumptive diagnosis of early complete miscarriage, but was admitted again on 19 December 1986 because of recurrent painless vaginal bleeding. The β-hCG level was 4920 m-i.u./ml Pelvic ultrasound examination showed a slightly enlarged uterus with no gestational sac, a right polycystic ovary, a normal left ovary and an enlarged left cornua but no fluid in the cul-de-sac. A repeat curettage was performed, and a small amount of tissue containing necrotic chorionic villi associated with blood clot was extracted from the left cornual region. An immediate laparoscopy was performed, because of the possibility of an interstitial pregnancy. The uterus was asymetrically enlarged by a swelling at the left cornu 3 cm in diameter, both tubes and ovaries were normal and there was no blood in the peritoneal cavity.

The laparoscopic appearance and the chorionic tissue, extracted by curettege from the left enlarged cornu, were highly suggestive of a left interstitial pregnancy. β-hCG levels during the days following the second curettage and the laparoscopy were 2340, 1720, 1680 and 2250 m-i.u./ml. Therefore, because of the evidence of trophoblastic activity and the probable difficulties of surgery we decided to use chemotherapy.

The patient received four courses of actinomycin-D, each course consisted of 0·5 mg given intravenously daily for 5 days. There was a 7-day interval between the treatment courses (Fig. 1).

During therapy, the haemoglobin level, white blood cell count, platelet count, liver function, renal function, and coagulation tests, as well as plasma electrolytes remained within normal limits.

Following the second treatment cycle, a hysterosalpingogram showed a filling defect in the left cornu (Fig. 2).

The β-hCG level decreased gradually and returned to below the upper limit of normal (<5 m-i.u./ml) after the fourth treatment cycle

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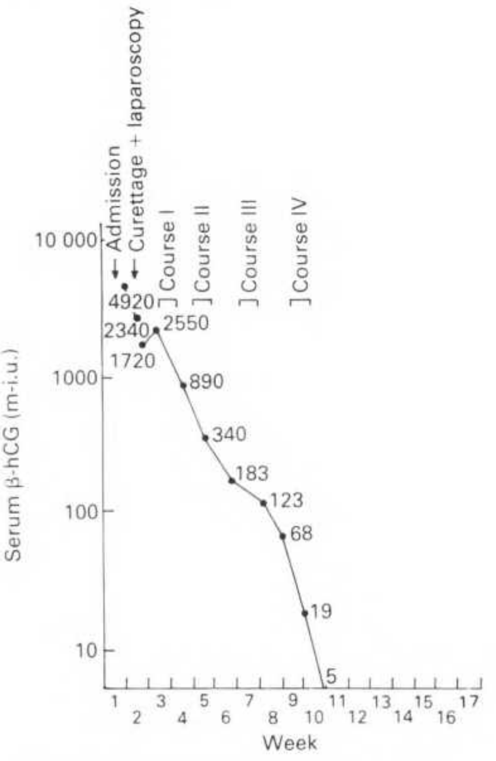

Fig. 1. β-hCG levels checked over several days.

(on 5 February 1987), and has remained negative until the present.

A second hysterosalpingography 6 weeks later showed an intact and normal left cornu (Fig. 3).

Discussion

The standard therapy for ectopic pregnancy is the surgical removal of the conceptus. In the past few years, medical treatment with cytotoxic drugs has been proposed as a possible alternative for women with unruptured ectopic pregnancies. Tanaka *et al.* (1982), followed by others (Brandes *et al.* 1986; Rodi *et al.* 1986; Farabow *et al.* 1983; Chotiner 1985; Miyazaki 1983), successfully used methotrexate for the non-surgical treatment of interstitial and tubal pregnancies. Methotrexate is effective as a chemotherapeutic agent in gestational trophoblastic disease. However, there are two major disadvantages to its use; there are major side-effects, such as bone marrow depression and stomatitis, and there is a possibility that there may be an increased frequency of congenital anomalies in future pregnancies (Walden & Bagshawe 1976), although some authors deny that methotrexate is responsible for the anomalies (Ross 1976).

Actinomycin-D has been used as an alternative chemotherapeutic agent for trophoblastic disease in the belief that it is less toxic than methotrexate (Disaia & Creasman 1981). Moreover, Rodi *et al.* (1986) reported seven women with unruptured tubal pregnancies treated with methotrexate followed by rescue with folinic acid, but they questioned whether there is a better agent than methotrexate to treat ectopic pregnancies medically. We preferred to use actinomycin-D as the therapeutic agent in the current case. The patient tolerated the treatment well, except for nausea which was successfully controlled with anti-emetic agents.

There are advantages in employing chemotherapy for unruptured ectopic pregnancies in locations where surgery is difficult and may result in hysterectomy. Examples of these are interstitial and cervical pregnancies. There may be a wider role for medical therapy for an ectopic pregnancy but, so far, there is insufficient evidence for the immediate and long-term safety of this method for it to be used routinely for unrup-

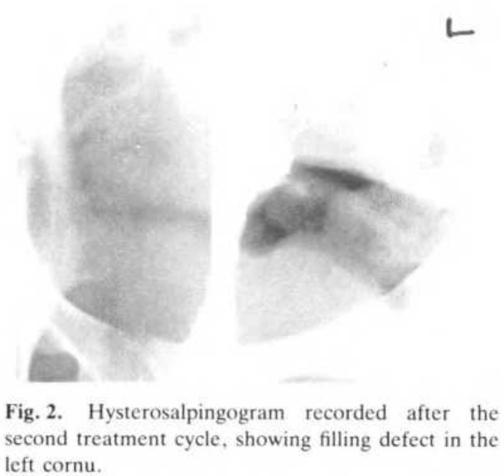

Fig. 2. Hysterosalpingogram recorded after the second treatment cycle, showing filling defect in the left cornu.

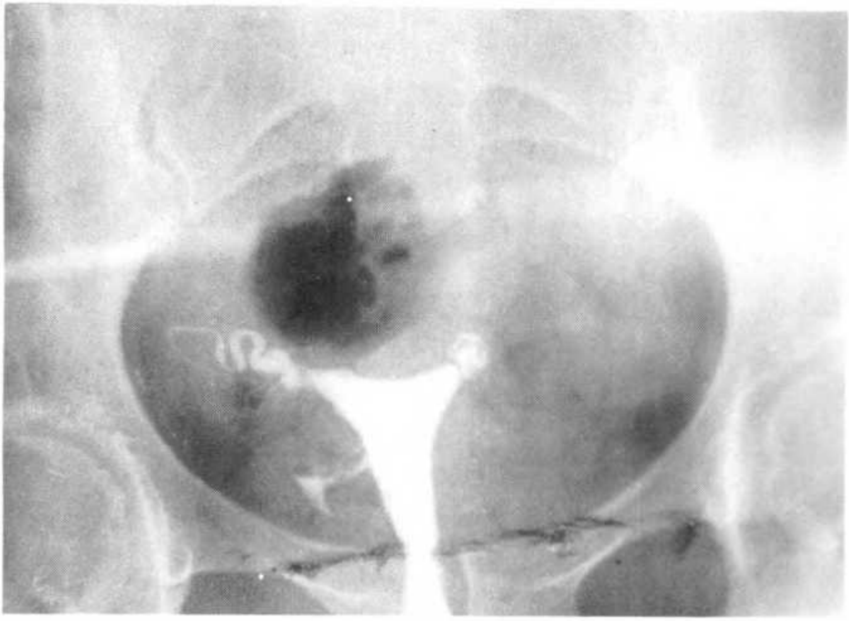

Fig. 3. Hysterosalpingogram recorded 6 weeks after the fourth (and last) actinomycin-D therapy, showing a normal left cornu.

tured tubal pregnancies. We consider actinomycin-D a reasonable alternative to methotrexate when the site of the ectopic pregnancy is such that surgical intervention might result in hysterectomy.

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Primary insular argentaffin carcinoma of ovary. Case report and literature review

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Case report

A 60-year-old woman presented in November 1983, with a 3-month history of increasing lethargy, exertional dyspnoea, bilateral ankle oedema and increasing facial colouration. She had a long history of mild reversible obstructive airways disease but had recently experienced periodic episodes of pronounced wheeze associated with facial flushing occurring approximately once per week. She denied gastrointestinal disturbance. Her past history comprised appendicectomy at 17 years of age, and right salpingo-oophorectomy for removal of a benign cystic teratoma at the age of 27 years; the latter was at 3 months gestation in the first of three otherwise uncomplicated pregnancies. She had undergone an uneventful menopause at 48 years of age.

Examination revealed marked violaceous discolouration of the forehead and cheeks which increased in intensity during the wheezy attacks, one of which was observed after admission to hospital. She was normotensive and the jugular venous pressure was elevated to 10 cm H_2O above the sternal angle with prominent V-waves; there was bilateral pitting oedema to the knees. A soft pan-systolic murmur was audible over the whole precordium, maximal at the left sternal edge during inspiration, and there was a widespread expiratory wheeze. The liver was palpable to 4 cm below the costal margin, and was tender; there was no clinical evidence of ascites. There was a cystic mass in the right iliac fossa, and on bimanual examination this was felt continuous with a solid component which filled the pelvis, lying behind a normal-sized uterus; the whole tumour was equivalent in size to a 16-week pregnancy.

Abdominal ultrasound confirmed the presence of a mass of mixed echogenicity arising from the pelvis, probably ovarian in origin; the liver, gall bladder and kidneys appeared normal. Intravenous urography revealed a soft tissue mass on the control film, with two teeth and several bony fragments; the kidneys were of normal size and excreted promptly; there was early clubbing of the calyces with ureteric dilatation down to the pelvic brim consistent with compression by the mass. An electrocardiogram showed evidence of right axis deviation although the chest radiograph showed no abnormality. Two-dimensional echocardiography showed a dilated right ventricle with paradoxical septal movement suggesting tricuspid regurgitation. Urinary 5-hydroxyindole acetic acid (5-HIAA) was elevated at 224 μmol/24 h (laboratory normal <52 μmol/24 h) and urinary hydroxymethyl mandelic acid was 60 μmol/24 h (laboratory normal <40 μmol/24 h) and urinary catecholamines were normal. A ventilation/perfusion scan showed patchy ventilation with washout markedly delayed; perfusion was also patchy, although there were no obvious mismatches. Additional investigations, including full blood count, plasma urea and electrolytes, proteins, liver function tests, blood gases, thyroid function, and isotope liver scan all showed normal results.

A provisional diagnosis of carcinoid syndrome with associated valvular dysfunction and right heart failure, secondary to an ovarian carcinoid tumour, was made, and laparotomy undertaken. At operation there were approximately 100 ml of free fluid in the peritoneal cavity which were

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sent for cytological examination. There was a large left ovarian tumour filling the pelvis, extending into the sigmoid mesentery and densely adherent to the uterus, the sigmoid colon, and the pelvic peritoneum; the most dependent parts of the tumour were solid, although a cystic component extended upwards to lie in the right iliac fossa. The uterus itself appeared normal, and the right ovary was absent. The liver had a grey appearance with a small thickened area on the upper surface of the right lobe, but without obvious tumour deposits. The diaphragm, omentum and peritoneal surfaces appeared healthy, as did the large and small intestine, although there were several mesenteric nodes palpable. Total abdominal hysterectomy, left salpingo-oophorectomy, omentectomy, liver biopsy and mesenteric node biopsy were performed. The patient suffered severe bronchospasm during manipulation of the tumour despite clamping of the infundibulopelvic ligament at the earliest opportunity. Her early postoperative care was therefore given in the intensive therapy unit; she returned to the routine ward after 12 h, and subsequent recovery was straightforward, indeed she expressed a profound sense of well-being from 24 h after surgery. Urinary 5-HIAA was checked on the 5th and 10th postoperative days and was negative on each occasion.

Pathology

Histological examination of the uterus, cervix, fallopian tube, omentum, liver and mesenteric nodes failed to demonstrate any abnormality. The left ovary was replaced by a tumour 210 × 130 × 85 mm with a smooth, bosselated external appearance. On section the tumour consisted of a thin walled cyst 110 mm in diameter, containing sebum, hair and teeth; there was a solid area at one end, 90 mm in maximum diameter, containing several small cysts and areas of necrosis (Fig. 1). Histological examination showed a carcinoid tumour with a predominantly insular pattern with one area showing acinar formation (Fig. 2); all areas demonstrated argyrophilia with Grimelius' stain (Fig. 3), and argentaffinicity when stained by the diazo technique; an infiltrative pattern of growth was seen at the edges of the tumour. Electron microscopy revealed the pleomorphic electron-dense secretory granules characteristic of an insular carcinoid (Fig. 4). Cytological examination of the peritoneal fluid showed erythrocytes, lymphocytes, histiocytes and groups of cells with small

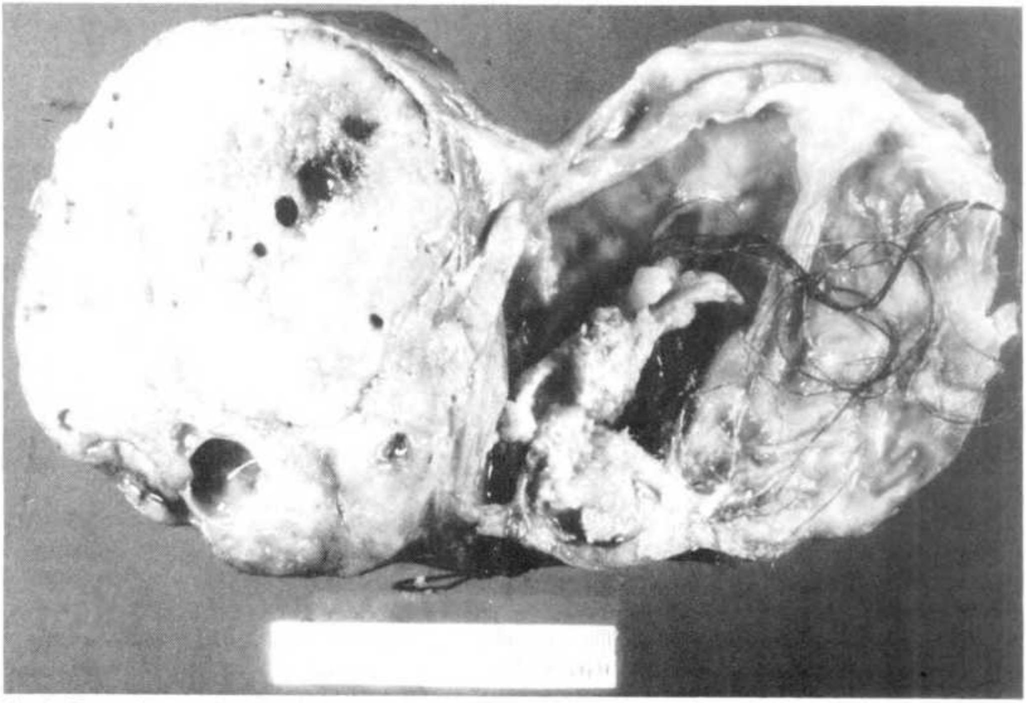

Fig. 1. Gross photograph of the left ovarian tumour in section.

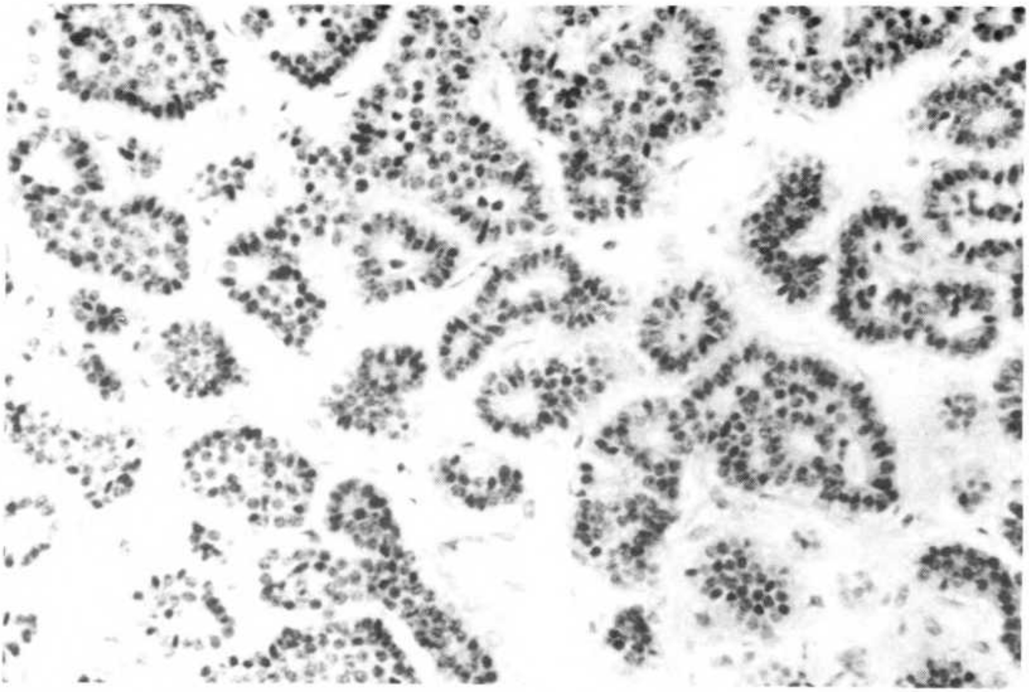

Fig. 2. Photomicrograph of the solid component of the tumour; demonstrating the insular pattern, with areas showing acinar formation; haematoxylin and eosin. ×135.

round hyperchromatic nuclei consistent with an argentaffin carcinoma (Fig. 5).

Sections of fresh tumour were assayed for 5-hydroxytryptamine (5-HT) and 5-HIAA, both

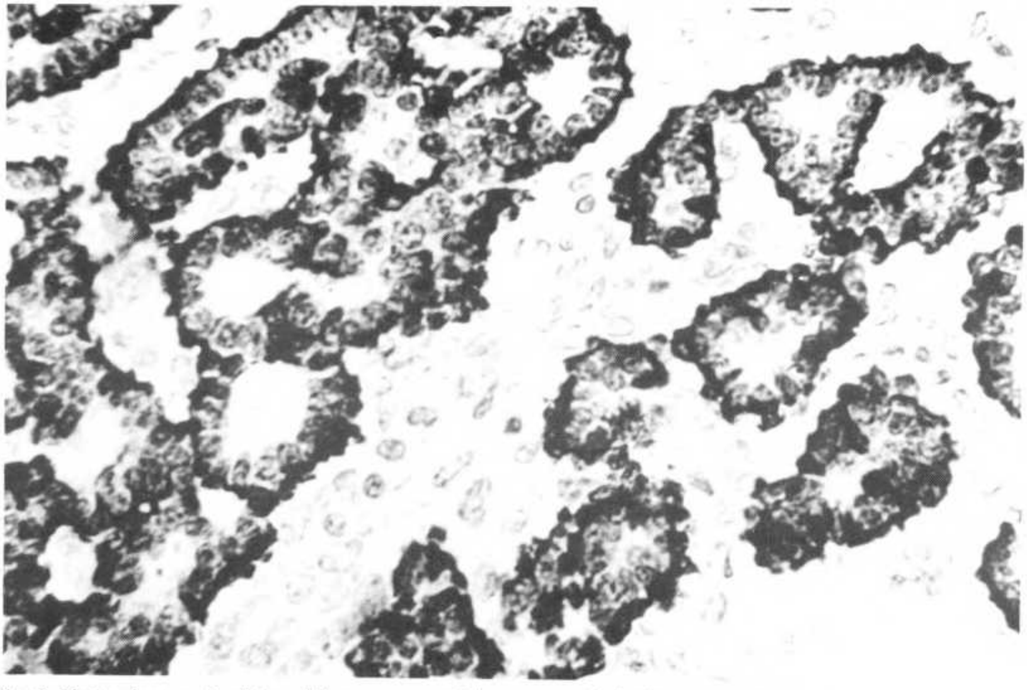

Fig. 3. Photomicrograph of the solid component of the tumour; Grimelius stain. ×270.

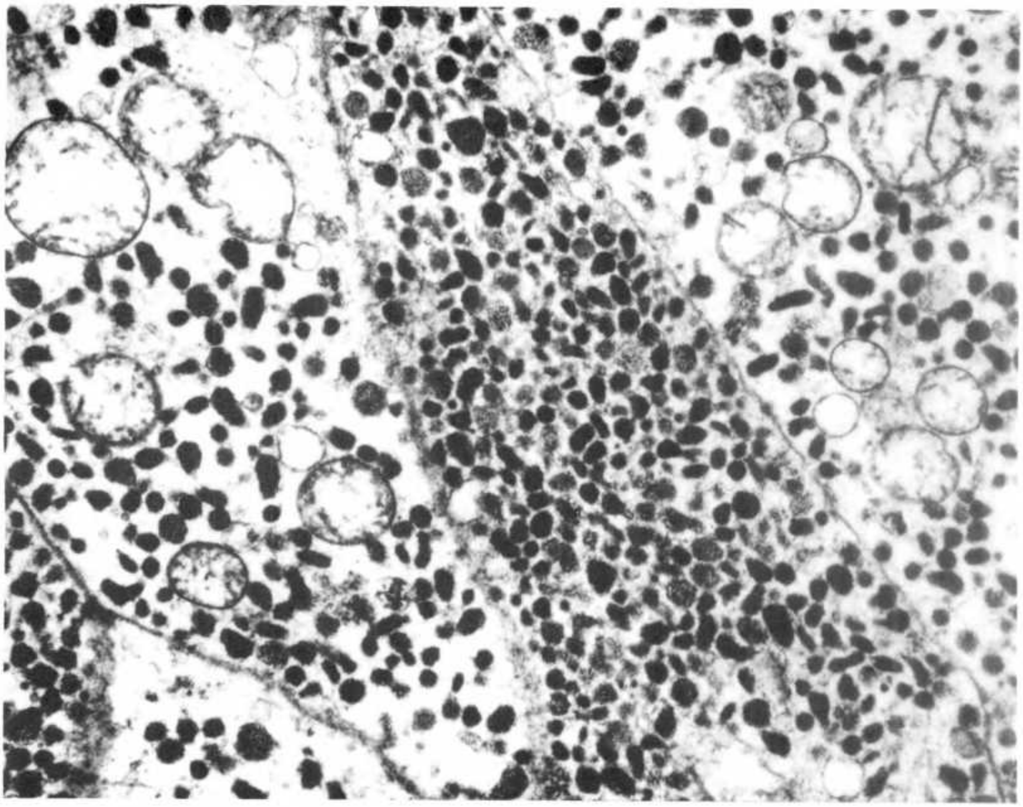

Fig. 4. Electronmicrograph demonstrating the pleomorphic electron-dense secretory granules separated from the limiting membrane. ×4560.

of which were grossly elevated at 0·7mg and 3·8 μg per gram of fresh tissue respectively. The endogenous 5-HT level was so high that a tryptophan hydroxylase assay was unsuccessful.

Follow-up

No adjunctive chemotherapy was given. Review was undertaken every 3 months during the first year after surgery, and annually since, the total follow-up period now being 4 years. Clinical assessment and urinary 5-HIAA estimation at each visit, and annual CT scanning have shown no evidence of tumour recurrence. Clinical and echocardiographic evidence of tricuspid regurgitation persists, although there has been no recurrence of congestive cardiac failure.

Discussion

The carcinoid tumour was first described by Lubarsch (1888), and the name coined by Oberndorfer (1907); it was not, however, until 1939 that the occurrence of carcinoid within an ovarian dermoid was recognized by Stewart *et al.* (1939), and as recently as 1954 that the carcinoid syndrome was described (Thorson *et al.* 1954). Primary ovarian carcinoids are rare tumours, accounting for fewer than 1% of all carcinoids (Hajdu *et al.* 1974); in an analysis of 2837 cases undertaken by Godwin (1975) only 3 (0·1%) were of primary ovarian origin. 'Pure' carcinoid tumours are found in the ovary (Kinley & Penner 1962; Ageev 1961; Haines 1971; Robboy *et al.* 1975; Holtz *et al.* 1979), and these have been regarded as arising either in argentaffin cells within the ovary (Toker 1969), or in migratory APUD cells (Trevensen *et al.* 1973; Sporrong *et al.* 1982); others have regarded them as a form of mono-dermal teratoma (Torvik 1960; Brown & Richart 1969). However, most arise within benign cystic teratomas (Adcock 1972; Pantoja *et al.* 1975), although they are encountered in fewer than 1% of ovarian dermoid cysts (Caruso *et al.* 1971; Robboy *et al.* 1975).

Histologically a variety of primary and meta-

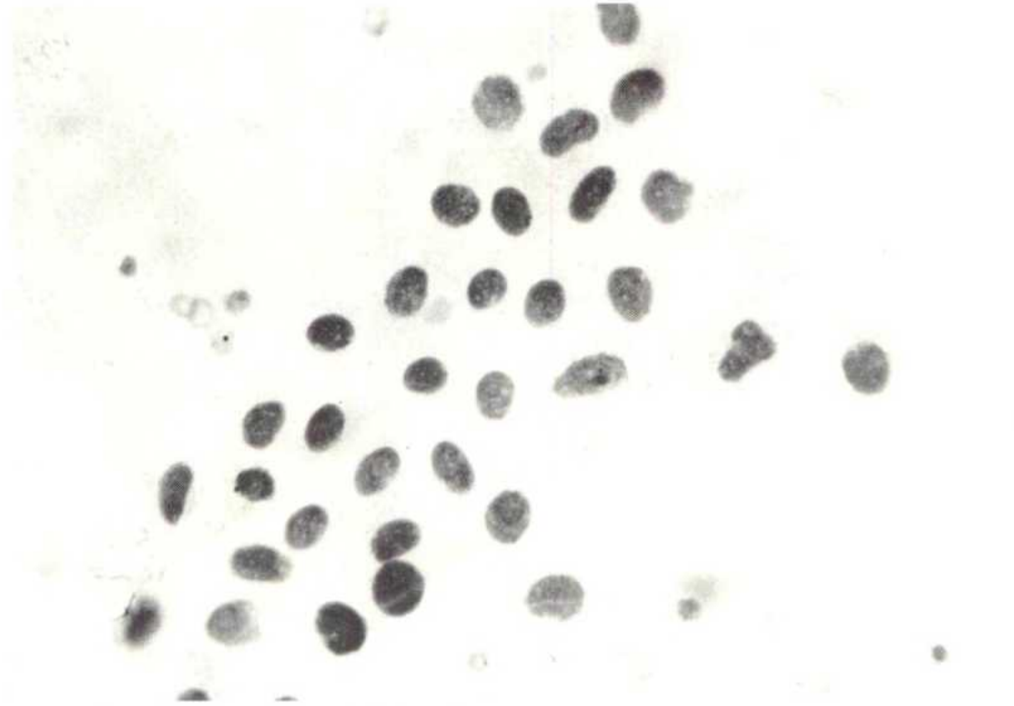

Fig. 5. Ascitic fluid; Papanicolou preparation. ×270.

static forms have been described. The most common, as in the case described, is composed of cells arranged in an insular pattern typically observed in carcinoids of midgut derivation (Robboy *et al.* 1975; Holtz *et al.* 1979). Less common is the trabecular form, which has a ribbon pattern typical of carcinoids arising in foregut or hindgut derivatives (Robboy *et al.* 1977; Talerman & Evans 1982). The trabecular pattern may be seen in an admixture with thyroid tissue, as a strumal carcinoid (Robboy & Scully 1972; Hart & Regezi 1978), and a mixed trabecular/insular pattern has been reported (Mitchell & Diamond 1949; Hensel & Decker 1964). Carcinoids metastatic to the ovary are almost always of small intestinal origin, and of the insular pattern (Robboy *et al.* 1974).

The heterogeneity of microscopic and histochemical features of carcinoids also extends to their ultrastructural characteristics, and in particular to the size and shape of secretory granules within tumour cells (Black 1968). Carcinoids of foregut derivation, with cells in a trabecular arrangement, are argyrophil-positive but only occasionally argentaffin-positive and contain small uniformly round granules (type I). Those of midgut derivation, with the insular pattern, contain large pleomorphic argentaffin-positive argyrophil-positive granules (type II). Finally tumours of hindgut derivation contain large uniformly round argentaffin-negative granules (type III). The ultrastructure of ovarian carcinoids has been investigated by Serratoni & Robboy (1975) and by Quizilbash *et al.* (1974). They confirmed the finding of pleomorphic granules within tumours of the insular type, corresponding to Black's type II granules. They varied greatly in size (90–410 μm), and often showed a reniform or dumb-bell shape; the electron-dense core was separated from the limiting membrane by a translucent zone. The appearances of the case reported here are entirely compatible with those descriptions (Fig. 4).

In common with carcinoid tumours of the gastrointestinal tract, ovarian carcinoids may secrete a wide variety of humoral agents. In an immunohistochemical study Sporrong *et al.* (1982) found immunoreactivity to a wide variety of neurohormonal peptides of gastroenteropancreatic origin in 53% of trabecular carcinoids and 42% of strumal carcinoids, but only 7% of insular carcinoids. The so-called 'carcinoid syndrome', however, is found only in association with tumours of the insular type, perhaps reflecting their more active secretion of 5-HT and vasoactive kinins, particularly bradykinin (Oates *et* *al.* 1966). Of the 75 cases of primary ovarian insular carcinoid previously reported in the world literature, approximately one-third have been associated with the 'carcinoid syndrome'. For the syndrome to arise, vaso-active substances must be released directly into the systemic circulation. Thus with gastrointestinal carcinoid tumours, symptoms only become manifest after metastasis to the liver has occurred. In ovarian lesions, however, the critical factor in determining symptom production appears not to be the development of metastasis, but simply the tumour size. In the cases reviewed by Robboy *et al.* (1975), the median diameter of those tumours associated with the syndrome was 9 cm as against 3 cm in those not associated with the syndrome.

Valvular dysfunction associated with 'carcinoid syndrome' occurs in up to 50% of cases, and heart failure as a consequence is a common cause of death (Hubbard 1986). It predominantly affects the right heart valves leading to tricuspid regurgitation and pulmonary stenosis, the latter being less common (Nielsen & Manners 1979). Right heart damage appears to occur as a result of repeated endothelial injury initiated by bradykinin release from the tumour (Ferran & Roberts 1976) leading to characteristic fibrotic plaques which restrict valve leaflet motion (Roberts & Sjoersma 1964). Two-dimensional echocardiography has been found to be useful both diagnostically and for following the progress of the condition (Howard *et al.* 1982). Cures in carcinoid heart disease are rare, although those cases due to a primary ovarian carcinoid tumour are potentially curable by removal of the primary tumour. Most such patients recover symptomatically, although, as in the case presented, physical signs usually remain. Progression occasionally occurs, although fortunately has been shown to be amenable to cardiac surgery (Stephan & de Wit 1974; Sworn *et al.* 1980).

Review of the literature suggests a favourable long-term prognosis for ovarian carcinoids; an actuarial survival of 95% at 5 years and 88% at 10 years was reported by Robboy *et al.* (1975). Evidence of malignant behaviour is rare, the first such case being reported by Saunders & Hertzman (1960); their patient showed no evidence of extra-ovarian tumour at initial surgery but died from hepatic metastases 5 years later. Chatterjee & Heather (1968) reported a similar case dying 6 years after presentation, and Sens *et al.* (1982) record a patient who died 3 weeks after admission with widespread metastases. Several other cases of supposed malignant ovarian carcinoid tumour have appeared in the literature (Brown & Lane 1965; Koven *et al.* 1968; Haines 1971), although subsequent review of these cases (Robboy *et al.* 1975) suggests that the diagnosis was other than carcinoid in the first two cases, and was inconclusive in the third.

Kinley & Penner (1962) and Robboy *et al.* (1975) recorded two cases where ascites was noted at operation, although in neither was cytological examination of the fluid undertaken. S. J. Robboy (personal communication) has on record a further six cases of primary ovarian insular carcinoids in which ascites was present at operation; three of these patients were alive and well at 3 months to 11 years after diagnosis, and one died from congestive heart failure after 4 years; two were diagnosed at post-mortem and in each the peritoneal surfaces were described as having a pearly-white coating resembling 'cake-icing'; again, in none of these cases was cytological examination available. Our patient would seem to provide the first report of malignant ascites in association with a primary insular argentaffin carcinoma of ovary. Because of the rarity of this clinical situation, there are no firm guidelines regarding the employment of adjunctive chemotherapy, although actinomycin-D (Koven *et al.* 1968), or vincristine, actinomycin-D and cyclophosphamide (Piver 1983) have been advocated. In our patient it was decided to withhold chemotherapy until such time as there was evidence of macroscopic tumour recurrence. The insensitivity of 5-HIAA production as a means of monitoring recurrence is shown by the following calculation:

Initial tumour 5-HIAA production = 224 μmol/24 h.

Volume of solid tumour component = 380 cm^3 —hence tumour 5-HIAA secretion = 0·6 μmol/cm^3/24 h.

Laboratory normal range for 5-HIAA = <52 μmol/24 h —hence 90 cm^3 functioning tumour would be required before significant increase in urine excretion could be expected.

Robboy *et al.* (1975) found the average 5-HIAA excretion rate among nine reports in the literature to be somewhat higher than this at 0·3 mg/cm^3/24 h (1·6 μmol/cm^3/24 h). Nevertheless, plasma 5-HT levels or CT scanning may be a more sensitive index of tumour recurrence. Obviously in view of the outcome in the small number of reports of malignant insular carcinoid tumours, continuing follow-up is mandatory.

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SHORT COMMUNICATIONS

Serum thyrotrophin concentration measured by sensitive assays in normal pregnancy

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The concentration of free thyroid hormones is reported to decrease (Giles 1982), increase (Wang *et al.* 1985) or remain unchanged (Symons *et al.* 1983) during pregnancy. Similarly basal thyrotrophin (TSH) has been reported to increase (Weeke *et al.* 1982; Pacchiarotti *et al.* 1986), decrease (Yamamoto *et al.* 1979; Harada *et al.* 1979) or remain normal (McLellan *et al.* 1987) in pregnancy. Here we report changes in serum TSH measured by six ultrasensitive assays in normal pregnancy.

Subjects and methods

Venous blood samples were collected from 75 pregnant women (age 19–32 years), 25 in each trimester, who were attending the antenatal clinic. Serum was separated and stored at −20°C. None of the pregnancies was complicated and none of the women was known to take any medication other than iron and vitamin supplements.

Blood samples taken from 40 non-pregnant healthy women (age 20–42) served as the reference values for comparison.

Serum total thyroxine (TT4) was measured by a fluorescence polarization immunoassay (Abbott TT4, T4 plus). Serum total T3, T3 uptake and free T3 were measured by radioimmunoassay using a commercial kit (Diagnostic Products Corporation). Free T4 was measured by a non-analog two-step immunoextraction assay (Gammacoat FT4, Clinical Assays, Dade). Serum TSH was measured using six different commercial sensitive assays: (A) Abbott RIABEAD TSH—an immunoradiometric assay, (B) Amersham Amerwell TSH, (C) Corning mab Magic TSH, (D) Hybritech Tandem-R TSH, (E) Boots Celltech Sucrosep TSH, and (E) Corning Magic Lite TSH–an immunochemiluminometric assay. The lower detection limits of these TSH assays in our hands were: (A) 0·03 m-i.u./l, (B) 0·06 m-i.u./l, (C) 0·12 m-i.u./l, (D) 0·07 m-i.u./l, (E) 0·18 m-i.u./l, (F) 0·02 m-i.u./l.

Results between groups were compared by Student's *t*-test and by the Mann–Whitney *U*-test.

Results and discussion

Table 1 summarizes the results. Serum TT4 concentration was highest in the second trimester. Serum T3 resin uptake was lower and the calculated free thyroxine index (FTI) was higher during the first two trimesters. Serum FT4 decreased throughout pregnancy and lowest values were seen in the third trimester. Changes in TT4 and FTI are in general agreement with results reported in the literature (Tolino *et al.* 1985; White 1987). However, serum FT4 has been reported to remain unchanged (Osthanondh *et al.* 1976; Symons *et al.* 1983), decrease (Gow *et al.* 1985) or increase (Wang *et al.* 1985) and some of the discrepancies are due to the different methods used in the assay (White 1987). The assay used for FT4 in this study has been reported to be correlated with the reference method—namely equilibrium dialysis (Jackson & Ekins 1986). Thus it is likely that there is a true fall in FT4 during pregnancy (Gow *et al.* 1985). Total T3 and FT3 were higher throughout pregnancy. The FT3 assay used is an analog method and it has been reported to give erroneous results in pregnancy (Witherspoon *et al.* 1988).

Serum TSH measured by all six assays showed a small but significant decrease in the first trimester (Table 1). Pacchiarotti *et al.* (1986), using the Boots Celltech assay, and Weeke *et al.*

Table 1. Serum thyroid hormone and thyrotrophin concentration in normal pregnancy

		Trimester of pregnancy		
	Healthy women (*n* = 40)	1st (*n* = 25)	2nd (*n* = 25)	3rd (*n* = 25)
Gestation (weeks)	—	9·4	18·5	34·2
Total thyroxine	105	145**	160**	139**
(nmol/l)	(66–145)	(78–212)	(110–210)	(89–189)
T3 uptake (%)	30·2	28·2**	24·7**	23·5**
	(22·6–37·8)	(23·2–33·2)	(20·1–29·3)	(18·5–28·5)
Free thyroxine index	104	126**	122**	101
	(65–144)	(78–174)	(76–168)	(70–132)
Free T4 (pmol/l)	15·8	13·7**	11·6**	9·5**
	(10·8–20·8)	(8·6–18·8)	(6·6–16·6)	(5·3–13·7)
Total T3 (nmol/l)	1·4	2·2**	2·4**	2·1**
	(0·8–2·0)	(1·1–3·3)	(1·6–3·2)	(1·2–3·0)
Free T3 (pmol/l)	2·7	3·9**	4·5**	3·4**
	(1·1–4·3)	(2·2–5·6)	(2·9–6·1)	(1·1–5·7)
TSH assays (m-i.u./l)				
(A) Abbott	1·15	0·70**	0·97	1·15
	(0·3–3·0)	(0–1·6)	(0·1–1·8)	(0·4–2·7)
(B) Amerwell	1·28	0·78**	1·02	1·12
	(0·4–3·5)	(0·1–1·7)	(0·3–1·9)	(0·5–2·5)
(C) Corning IRMA	1·26	0·73**	1·23	1·45
	(0·4–3·2)	(0–1·8)	(0·2–1·9)	(0·4–2·3)
(D) Hybritech	0·98	0·61**	0·83	0·85
	(0·3–2·8)	(0–1·5)	(0·2–1·8)	(0·4–2·4)
(E) Boots	1·75	1·00*	1·31	1·65
	(0·4–4·9)	(0–2·4)	(0·2–2·5)	(0·8–4·6)
(F) Corning ICMA	1·15	0·66**	1·01	1·19
	(0·3–3·2)	(0–1·8)	(0·1–2·3)	(0·4–2·8)

Results are given as mean, and range in parenthesis.

Significantly different compared with healthy non-pregnant women $^{*}P<0{\cdot}05$; $^{**}P<0{\cdot}01$.

(1982), using a conventional TSH assay with a detection limit of 0·2–0·4 m-i.u./l, showed an increase in TSH during pregnancy. On the other hand, Harada *et al.* (1979) and Yamamoto *et al.* (1979) found a significant decrease in serum TSH at 9–12 weeks. We observed a consistent decrease in TSH during the first trimester by all six assays and it is unlikely that this low TSH is due to cross-reactivity with human chorionic gonadotrophin (hCG) as these assays used two highly specific monoclonal TSH antibodies. The observation that TSH response to TRH is suppressed in the first trimester of pregnancy (Guillaume *et al.* 1985) further confirms our observation. The suppression of TSH in the first trimester is likely to be due to hCG which is highest at 8–11 weeks (Yamamoto *et al.* 1979), and has some TSH-like activity (Harada *et al.* 1979; Braunstein & Hershman 1976).

We conclude that there is a significant decrease in FT4 during pregnancy and a significant suppression of TSH during the first trimester of pregnancy.

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Haemodynamic changes during early pregnancy loss

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The increase in cardiac output during pregnancy is thought to begin early in the first trimester (Atkins *et al.* 1981; Robson *et al.* 1987*a*). The haemodynamic changes in subjects with early pregnancy loss have not been previously reported.

Subjects and methods

Seventeen healthy women were originally recruited before conception as part of a prospective study of maternal haemodynamics during early pregnancy. Four pregnancies miscarried in the first trimester in three women [A (two pregnancies), B and C]. Thirteen women who had uncomplicated pregnancies were used as controls. Haemodynamic data from 12 of this group have been reported previously (Robson *et al.* 1987*a*). Investigations were performed before conception and then at 5, 8 and 12 weeks of pregnancy, confirmed by β-human chorionic gonadotrophin (hCG) assay, or until the subjects miscarried. Investigations were performed using an echocardiographic Doppler system (Hewlett Packard 77020A) with the subjects in the left semi-lateral position after a minimum of 15 min rest.

Cardiac output was measured by combined Doppler and cross-sectional echocardiography. Velocity and cross-sectional area measurements were obtained from the aorta, pulmonary artery and mitral valves (Robson *et al.* 1987*b*) and the mean flow calculated. The results of the control group were analysed by a repeated measures analysis of variance.

Results

The means and within-subject 99% confidence intervals (CI) for the normal group are shown in Fig. 1. In two pregnancies, the first in A and B, which miscarried between 5 and 8 weeks, no haemodynamic change was apparent at 5 weeks. In pregnancy C an apparently normal haemodynamic response occurred until 8 weeks. Although an ultrasound scan confirmed a live fetus, the patient was admitted at 10 weeks gestation with an inevitable miscarriage. In the second pregnancy of patient A, stroke volume increased markedly between 5 and 8 weeks. An ultrasound scan at 12 weeks showed a gestation sac but no fetal pole. Interestingly, between 8 and 12 weeks cardiac output and stroke volume had fallen quite dramatically.

Discussion

Our results confirm that the changes in cardiac output began early in the first trimester along with many of the other major pregnancy adaptations (Hytten 1968). Minimal haemodynamic changes were observed in the two very early miscarriages (<8 weeks). In contrast, a normal haemodynamic response was seen where a live fetus was identified at 8 weeks gestation. The haemodynamic changes seen with a blighted ovum are perhaps the most interesting. After an initial increase, stroke volume and cardiac output fell between 8 and 12 weeks when clinically the pregnancy appeared to be progressing satisfactorily.

The factors responsible for these haemodynamic changes are uncertain. There is some evidence to suggest that hCG concentrations relate closely to alterations in renal physiology during early pregnancy (Davison *et al.* 1988). However, no clear temporal relation has been demonstrated between changes in hCG concentrations and early pregnancy failure (Aspillaga *et al.* 1983). On the other hand, oestradiol and progesterone concentrations have been shown to increase and then fall in cases of blighted ova, the decrease generally occurring after 8 weeks gestation (Aspillaga *et al.* 1983). Oestrogens are known to increase stroke volume and cardiac output in man (Slater *et al.* 1986) and the changes reported here support the suggestion that oestrogens are responsible for the haemodynamic changes during pregnancy (Longo 1983).

Acknowledgment

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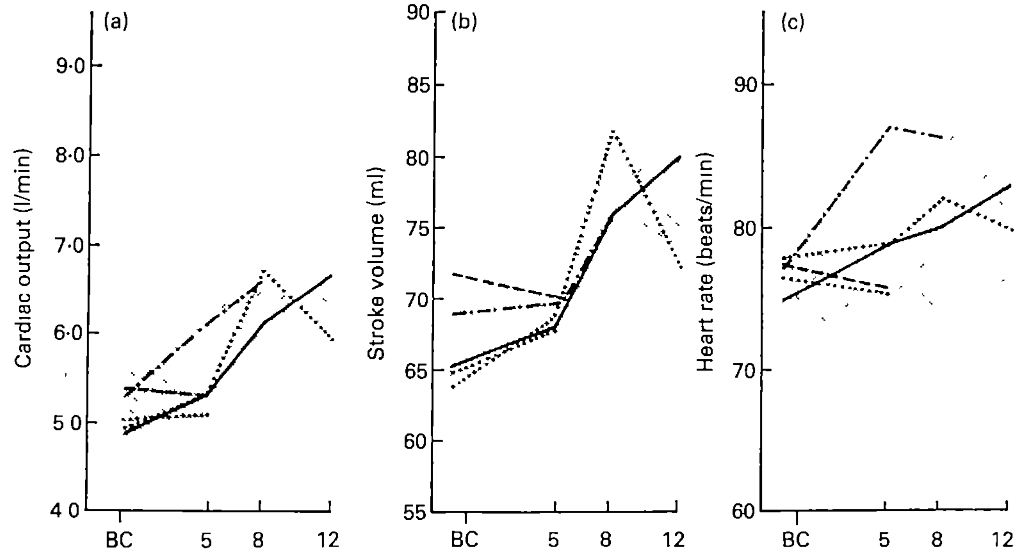

Fig. 1. Cardiac output (a), stroke volume (b) and heart rate (c) before conception (BC) and during the first trimester of pregnancy. The mean and 99% confidence intervals for the normal group are shown as a solid line and stippled area. Subject A (two pregnancies) (· · · ·), subject B (——), subject C (–·–).

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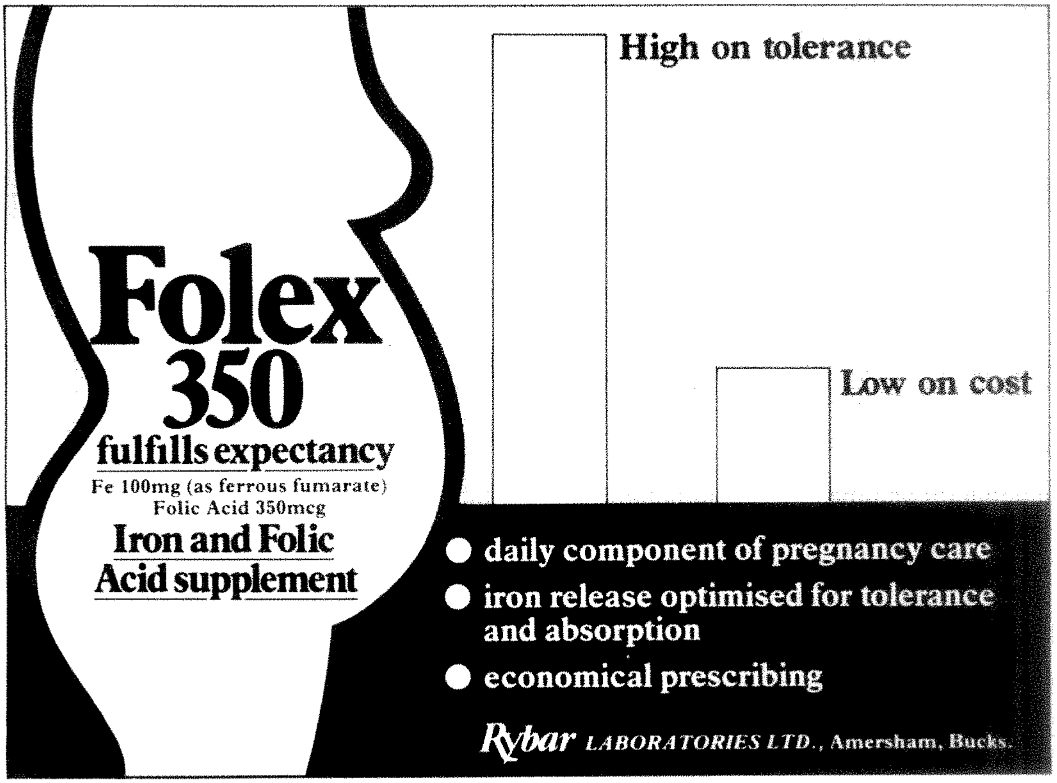

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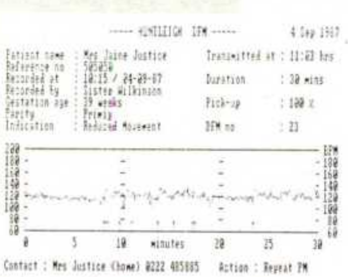

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Reference no   : 505050
Recorded at    : 10:15 / 04-09-87       Duration       : 30 mins
Recorded by    : Sister Wilkinson
Gestation age  : 39 weeks               Pick-up        : 100 %
Parity         : Primip
Indication     : Reduced Movement       DFM no         : 23

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British Journal of Obstetrics and Gynaecology
December 1988, Vol. 95, pp. 1337–1341

CORRESPONDENCE

Disposal arrangements for fetuses lost in the second trimester

Dear Sir,
The paper by Batcup *et al.* [*Br J Obstet Gynaecol* (1988) **95**, 547–550] and the accompanying commentary by Morris [ibid. 545–546], rightly drew attention to ways in which parents may be helped to deal with second trimester fetal loss, whether spontaneous or following elective termination. Morris (1988*b*) has recently written in similar vein concerning perinatal loss. These authors point out that appropriate 'disposal' of fetuses may well be of great help to the families, while Morris also touches on the issue of providing counselling support for parents in attempting to prevent subsequent mental illness in the family.

I take the view that the foundation for all these helping modes, that may speed recovery from fetal loss, is the provision of hard facts about what actually happened to the pregnancy, and what is the likelihood of recurrence. I am always surprised at the apparent speed and relative equanimity with which parents come to terms with fetal loss. In the case of selective termination for fetal anomaly, the parents usually manage to make a rapid U-turn from a wanted pregnancy to one that they may decide to terminate. The complexities of ultrasound, cytogenetics, alphafetoprotein and a range of fetal anomalies have to be discussed, often against a time constraint because of gestational age. Proper leave-taking of the fetus may clearly be extremely helpful, but it should not be the exclusive concern, because of the longer term issues that remain to be discussed and resolved.

Among the multitude of caring services that the parents can receive, a thorough fetal mininecropsy does not often appear at the top of the list of priorities, and is seldom mentioned as a major means of assuaging parental feelings of guilt, helplessness and apprehension about future reproduction. Maybe it is taken for granted, but it should not be. In my experience, it is these hard, factual data, when available, that are very useful in setting minds at rest, and, sometimes, preventing litigation. I can quote from a letter I recently received from a woman who lost monozygotic, monochorionic twins at 19 weeks gestation with twin transfusion syndrome and severe acute polyhydramnios: 'It is definitely of prime importance to find out if there was a genetic deficiency . . . Hence, an autopsy is important, even if a parent may be opposed, because it is the only way one can answer the question—Why?'

I think it would be useful to have long-term follow-up studies to compare the outcomes for parents who do and do not receive factual information about fetal/placental pathology. In the same way that the prenatal diagnosis and management of fetal loss may be multidisciplinary (Machin *et al.* 1987; Porter *et al.* 1988), it seems to me that postnatal care for parents could use many modalities of care, from the most scientific to the most pastoral, starting with the 'sympathetic pathologist' (Benirschke 1987).

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Sexually transmitted diseases including genital papillomavirus infection in male sexual partners of women treated for cervical intraepithelial neoplasia III by conization

Dear Sir,

Bistoletti & Lidbrink [*Br J Obstet Gynaecol* (1988) **95**, 611–613] have examined male partners of women treated 3 years previously for CIN III. That their study population appeared to have a high past history of sexually transmitted diseases (33%) is of note—there were no control data for comparison. This incidence is reasonably presumed to reflect previous sexual promiscuity. If these men had indeed been promiscuous, it is hardly surprising that they show evidence of another sexually transmitted disease, namely genital warts.

They have diagnosed human papillomavirus infection (HPV) by a number of criteria, including the presence of acetowhite epithelium. Acetowhite epithelium in males (as in females) is not a specific marker for HPV infection—indeed in half of their biopsies taken from such lesions (13 of 26) there was no histological evidence of HPV infection. With this in mind it would appear that none of the variables in their first Table reaches statistical significance. Their data thus fail to support the hypothesis proposed that the presence of HPV infection in male partners is a causal factor in the recurrence of CIN.

Generally there is a low rate of recurrence of CIN after adequate local destructive therapy. If HPV infection in male partners (with an incidence of more than 50% in this study) leads to recurrent disease then why do we not see a much greater recurrence rate?

Before suggesting that it is important to treat these areas of acetowhite epithelium, we need to answer several questions. Are these areas HPV infection? What is their significance for female partners? What is their natural history? And is there any acceptable treatment which eliminates subclinical HPV infection in man?

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Reference

Bistoletti, P. & Lidbrink, P. (1988) Sexually transmitted diseases including genital papillomavirus infection in male sexual partners of women treated for cervical intraepithelial neoplasia III by conization. *Br J Obstet Gynaecol* **95**, 611-613.

Authors' reply

Dear Sir,

We agree with Dr Griffiths on the importance of internal controls. Nevertheless, the use of external controls may be justified under certain circumstances (Bailar *et al.* 1984). We compared the partners of women with and without recurrent CIN. The regular sexual partners of the latter group were regarded as a control group.

The present study was inspired by the results of Campion *et al.* (1988) who found that only 3 of 25 (12%) male sexual partners of women without CIN had genital acetowhite lesions. We agree with Dr Griffiths that the acetic acid test may be an unspecific marker for the presence of HPV infection and that other conditions might cause acetowhite lesions. So far no studies have been published that allow the assessment of the sensitivity and specificity of the acetic acid test in the male.

The histopathological diagnosis of subclinical genital HPV infection in the male is also rather difficult, being subject to great inter- and intra-observer variation. The fact that we could not confirm a HPV infection histopathologically in all biopsies does not rule out an HPV infection.

Little is known about HPV transmission and contagiousness, in particular in patients with acetowhite epithelium or Bowenoid papulosis. Little is also known about the clinical significance and natural history of these lesions. We found recurrent CIN after conization for CIN III in 9% of 150 women, acetowhite lesions on the cervix in 45% and koilocytosis in 61% of the cervical biopsies. This suggests that neoplastic transformation is a very selective process.

The comments made by Dr Griffiths are highly relevant and illustrate the clinical difficulty in making a correct diagnosis of subclinical genital HPV infection in the male. Simple, non-invasive and sensitive virological methods are needed for future research. Our hypothesis is that genital HPV infection of the regular male sexual partner may play a causal role in CIN that recurs after treatment. We suggest that assessment and treatment of the male (or the use of barrier methods of contraception) may be of value in further reducing the frequency of recurrent CIN and cervical cancer. This can only be proven in prospective randomized clinical trials.

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Commentary: An oxytocin inhibitor for suppressing preterm labour

Dear Sir,

I share Professor Turnbull's enthusiasm [*Br J Obstet Gynaecol* (1987) **94,** 1009–1011] for the preliminary findings of Åkerlund *et al.* (1987) on the ability of the oxytocin antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin to inhibit the uterine contractions of premature labour. Certainly a tocolytic agent with a new mechanism of action would be a valuable addition to the obstetrician's armamentarium, despite the limitations of tocolytic therapy in general.

However, enthusiasm about the therapeutic value of such compounds must not lead to inaccuracies about the mechanisms involved. The hypothesis (Fuchs *et al.* 1982, 1984) that both normal labour and preterm uterine contractions can be explained by an increase in uterine oxytocin receptors is simple, attractive, almost universally accepted, but stands on very shaky ground. Evidence, obtained mainly from animal experiments, that throws doubt upon this hypothesis continues to mount and continues to be ignored, particularly in the pages of prestigious journals.

First and of most importance, there is considerable doubt as to whether the binding sites that have been claimed to change really are the physiological receptors for oxytocin (Pliška *et al.* 1986; Pliška 1988). Second, three independent studies (Goren *et al.* 1980; Crankshaw 1987; Tuross *et al.* 1987) have shown that the uterus *in vitro* does not behave as predicted by changes in the number of binding sites.

Third, exogenous manipulation of the hormonal environment was shown (Ruzycky & Crankshaw 1988) to produce a significant change in the number of oxytocin binding sites but not in either the in-vitro contractile response or the production of second messenger involved in the hormone's action on the rat's uterus. Finally the methods used in the studies on which the hypothesis is based (Fuchs *et al.* 1982, 1984) are not rigorous enough (Pliška *et al.* 1986) to support the hypothesis. As an example, similar methods were used to suggest that oxytocin and vasopressin act at the same site in the human endometrium (Fuchs *et al.* 1985) whereas more recent work clearly suggests that this is not the case (Guillon *et al.* 1987).

Perhaps subsequent experiments will provide explanations for these awkward observations that are compatible with the original hypothesis; at present, however, this seems unlikely. A duty to the well-being of their patients requires obstetricians to take a much more critical approach to currently available scientific information, whether they are therapeutic nihilists or not.

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Authors' reply

Dear Sir,

We appreciate Dr Crankshaw's comments on Professor Turnbull's commentary. The oxytocin analogue used by Åkerlund *et al.* (1987), l-deamino-2-Tyr-(OEt)-4-Thr-8-Orn-oxytocin, was chosen because of its potency as an inhibitor of oxytocin-induced contractility in human pregnant myometrium *in vitro* (Melin *et al.* 1986). The analogue had no intrinsic agonist effect. The in-vitro data and the preliminary in-vivo results (Åkerlund *et al.* 1987) showed that it was an oxytocin antagonist which could be useful in uncomplicated preterm labour. Further work is necessary to clarify the action of the analogue on oxytocin binding to human pregnancy myometrium and the generation of inositol phosphates as second messengers (Schrey *et al.* 1986). The analogue is also a potent inhibitor of vasopressin action on human non-pregnant myometrium (Melin *et al.* 1986), so it seems to be both an oxytocin and vasopressin receptor antagonist. This also needs to be confirmed by studies of binding and second messenger generation.

The increase in the sensitivity of the human uterus to oxytocin with advancing gestation (Caldeyro Barcia & Sereno 1961) and in preterm labour (Takahashi *et al.* 1980) is probably the result of increased oxytocin receptor concentration (Fuchs *et al.* 1984) and maturation of the signal transduction mechanism (Michell 1987), together with increases in the functional and catalytic proteins necessary for the contractile process (Matsui *et al.* 1983) and gap function formation (Garfield *et al.* 1980). We agree with Dr Crankshaw that measurement of oxytocin binding sites in crude myometrial membrane preparations does not provide a full picture, but the findings of Fuchs *et al.* (1984) agree with the clinical and pharmacological observations quoted above.

In Dr Crankshaw's letter, the references to oxytocin and vasopressin receptors 'in human endometrium' are not clear, and the papers he quotes refer to myometrium. Oxytocin receptors have been described in human and sheep endometrium (Fuchs *et al.* 1985; Flint *et al.* 1986) and in human decidual cells (Fuchs *et al.* 1984). In those tissues oxytocin provokes prostaglandin and arachidonic acid release, probably by stimulating phosphoinositide hydrolysis (Fuchs *et al.* 1982; Flint *et al.* 1986; Schrey *et al.* 1986; Wilson *et al.* 1988). Prostaglandins may enhance the sensitivity of the uterus to oxytocin (Riemer *et al.* 1986).

It is obvious that the actions of oxytocin on the human uterus are complex and more basic research needs to be done. A more complete picture should emerge when evidence from animal experiments is established in human material. Nevertheless, whatever biochemical mechanisms may be involved, the new analogue described by Dr Åkerlund and his colleagues seems promising for the management of uncomplicated preterm labour because of its apparent effectiveness, safety and freedom from side-effects, and deserves to be assessed in randomized controlled clinical trials.

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BOOK REVIEWS

Obstetric Fistula by Robert F. Zacharin. Springer-Verlag, Vienna, New York, 286 pages with figures and tables, DM230

Robert Zacharin, with characteristic thoroughness, has assembled a comprehensive history of the surgery of obstetric fistula to the present day. This is a condition which inspires interest tinged with awe in many gynaecologists. The reasons are not difficult to define. The condition is very rarely seen in communities with well-developed maternity services but, by contrast, in certain developing countries the surgery of obstetric fistula is still the major problem in clinical gynaecology. To this day individual surgeons in selected countries have acquired enormous experience and expertise. This book is dedicated to two expatriate Australasians with their own fistula hospital, while recognizing the contributions of others particularly in West Africa. The technical features reported are therefore slanted to the work of this group, which has otherwise not been reported.

Inevitably, therefore, the emphasis has been gynaecological and vaginal; the abdominal (and urological?) aspects, which are an important part of the armamentarium of the virtuoso, have been covered but less thoroughly—Swift-Joley does not rate a mention.

The last 40 pages are devoted to rectal fistulae. Although only one-tenth as common as obstetric vesico-vaginal fistulae, high rectal fistulae present a formidable surgical problem, often concurrent with a major bladder injury. In both situations segmental circumferential loss of the adjacent viscus may occur, and, although touched on, the complexity of this feature is not brought out in the text.

In all, this is an excellent historical review which will remain an authoritative source of data. It is also a good vicarious account of the Fistula House of the Princess Tsahai Hospital and a fitting tribute to its founders. For these reasons it is not also a *vade mecum* in the field.

Genital fistulae lie on the anatomical interface between subspeciality disciplines. It is tempting to those who have not been exposed to these problems to defer to the views of the specialist of the adjoining discipline. The message, however, from this work is clear. Obstetric fistulae are the proper province of the obstetrician and gynaecologist, and the experienced operator achieves better results than the amateur. The would-be subspecialist in gynaecological urology would be advised to add the full repertoire involved here to a wide operative experience in more complex surgical gynaecology.

G. N. Hudson

Decision Making in Infertility edited by Alan H. DeCherney, Mary Lake Polan & Stephen P. Boyers. B. C. Decker, Inc., Toronto, Philadelphia, 215 pages with figures and tables, £33.45

People affected by subfertility expect a coherent approach to their problem and are quick to detect uncertainty on the part of inexperienced staff or a lack of direction in their management. This useful book may help improve the management confidence and consistency of junior doctors faced with such patients and should make established specialists reflect on their own decision making. The authors provide a systematized approach to these issues based on the practice of the Yale University Medical School. Each aspect of management from the first interview through to surgical, medical and assisted reproduction treatments is dissected and presented in algorithm format. This gives a branching structure with one algorithm leading to another and so on. There are 104 such charts in total. Each is intended to be self-sufficient but the facing page is used to enlarge on some points from the chart on the opposite page.

In general the approach is well balanced but it would be surprising if one did not disagree with some aspects of management. For example in British practice culdocentesis does not generally find a place in the standard work-up of suspected ruptured ectopic pregnancy and I have yet to encounter a National Health Service histology service which routinely provides the detailed luteal-phase endometrial assessment employed in the American protocol. These matters reflect differences in practice but I was concerned that the section on 'ovulation induction gonadotrophin releasing hormone' was misleading. The authors cite failure to ovulate in response to pergonal as an indication for pulsatile GnRH therapy. This is illogical since pergonal-unresponsive ovaries would not be expected to respond to endogenous gonadotrophins stimulated by GnRH. On the same page the reader is advised to stimulate ovulation by means of pulsatile subcutaneous GnRH analogue (20–40 μg every 120 min) until an adequate follicular response is achieved. If this suggestion is followed the likely outcome would be a transient ovarian response then inactive ovaries unless the reader realizes that the text should probably replace 'GnRH analogue' by 'GnRH'.

The breaking down of all infertility practice into step-by-step charts is an interesting exercise and could be particularly helpful to MRCOG candidates who feel lacking in sufficient direct infertility experience since the approach used clarifies how everyday decisions are reached. It would be fascinating to see these management charts joined together on a wallchart which might be in the style of the metabolic pathway charts used by biochemists. No doubt in time an enterprising drug firm will take up the idea.

D. H. Barlow

International Strategies for the Eradication of Carcinoma of the Cervix in Developing Areas (Proceedings of the International Working Party Meeting in India, 1987) edited by R. F. Mould & Arabinda Bose. Adam Hilger, Bristol, 1987, 293 pages with figures and tables, £35.00

This book consists of the 58 papers presented at the Ninth meeting of the International Working Party in 1987, divided up by the nationality of the authors. These papers are prefaced by a useful summary of the most recently available worldwide rates of cervical cancer incidence, and have as a postscript a report of the guest lectures and ensuing discussion at the meeting. Unfortunately, the standard of the papers varies as much as the different typefaces in which they are printed. The subjects covered range widely from epidemiological studies of the aetiology of cervical cancer, to strategies for its prevention, to methods of treatment, with the major emphasis on details of particular radiotherapy techniques. Such variety reduces the chances of repetition, although several of the Indian epidemiological papers share similar introductions and discussions. Despite these problems of presentation, many of the results are interesting, such as the study from Calcutta showing that in a community in which women do not smoke cigarettes the rates of cervical neoplasia are higher in women who consume tobacco leaf products. Moreover, the papers highlighting the problems of screening women in the rural parts of developing countries serve to put the problems of our own system into perspective and demonstrate the need for better patient education as well as organization of services. Of the four papers from the UK, two present detailed descriptions of radiotherapy techniques and two discuss the use of radiotherapy in the treatment of endometrial cancer.

Despite the editorial stating that the book is aimed at 'developing countries in particular' and the inclusion of several advertisements for radiotherapy equipment, the price is more in the textbook range. Overall, even for the most interested reader, there is too much chaff amongst the grain to justify the purchase of this book.

S. E. Barton

Books received some of which may be reviewed at a later date

Principles of Obstetrics by Bryan M. Hibbard. Butterworths, London, 1988, 818 pages with figures and tables, £55.00

Gynaecological Endoscopy by Alan G. Gordon & B. Victor Lewis. Chapman & Hall, London, 1988, fully illustrated with colour plates, £45.00

Pregnancy and Risk. The Basis for Rational Management edited by D. K. James & G. M. Stirrat. John Wiley & Sons, Chichester, 1988, 209 pages with figures and tables, £18.50

Lecture Notes on Gynaecology Sixth edition by Josephine Barnes & Geoffrey Chamberlain. Blackwell Scientific Publications, Oxford, 1988, 270 pages, £9.95

Obstetrics and Gynaecology Revision by David M. Farquharson. Churchill Livingstone, Edinburgh, 1988, 235 pages with figures and tables, £9.50

A Practical Guide to Labour Management by Donald Gibb. Blackwell Scientific Publications, Oxford, 1988, 152 pages with figures and tables, £9.50

Atlas of Gynecologic Pathology by J. D. Woodruff & T. H. Parmley. Gower Medical Publishing, London, 1988, fully illustrated with colour plates, £60.00

Obstetric Anesthesia by Sivam Ramanathan. Lea & Febiger, Philadelphia, 1988, 420 pages with figures and tables, $45.00

Biopsy Pathology and Cytology of the Cervix by D. V. Coleman & D. M D. Evans. Chapman & Hall, London, 1988, 396 pages fully illustrated, £48.50

Multiple Sclerosis: Exploring Sickness and Health by Elizabeth Forsythe. Faber & Faber, London, 1988, 147 pages, £4.95 (UK), $8.95 (USA), $12.95 (Canada)

Contemporary Obstetrics and Gynaecology edited by Geoffrey Chamberlain. Butterworths, London, 1988, 434 pages fully illustrated, £27.50

Proceedings and Symposia

The Onset of Labor: Cellular and Integrative Mechanisms (Reproductive and Perinatal Medicine IX) A National Institute of Child Health and Human Development Research Planning Workshop, Bethesda, 1987, edited by D. McNellis, J. R. G Challis, P. C MacDonald, P. W. Nathanielsz & J. M. Roberts. Perinatology Press, Ithaca, 1988, US $57.50 (list price $68.50)

Erratum

Br J Obstet Gynaecol **95**, 1111–1116

Rapidly progressive cervical cancer: is it a real problem?

P. B. S. SILCOCKS & S. M. MOSS

On page 1112, right-hand column, line 6 *should* read: $B = 1 -$ sensitivity of the screening test (i.e. the false-negative rate).

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Authors should describe clearly the design of their investigation and the source of the data. All statistical methods should be specified so that it is clear which was used where Papers omitting this information may be returned without review The basic principle to follow is to supply sufficient information about the design and methods of analysis to allow the research to be repeated by someone else.

Some points of detail to note are

(1) It is not acceptable to present the results of a statistical analysis solely by a *P* value—actual results such as means or proportions should always be given, along with the test statistic.
(2) The use of confidence intervals is encouraged
(3) The symbol ± should not be used when presenting SE or SD. Presentation such as 'the mean . . . was 14·2 (SD 3·2) mg' is preferred
(4) When paired comparisons are made it is desirable to indicate the variability of within-pair differences
(5) Unusual methods of analysis should be described and/or referenced.

Authors are referred to the general statistical guidelines of Altman *et al* (1983) for advice about the presentation and interpretation of the statistical aspects of their research

Altman, D. G., Gore, S M , Gardner, M. J & Pocock, S J (1983) Statistical guidelines for contributors to medical journals *Br Med J* **286,** 1489–1493

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Manuscripts should contain a statement that the work was approved by the ethics committee of the institution in which it was done and that subjects gave informed consent to the work The editors reserve the right to reject papers if they consider the ethical justification to be questionable

References

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Trudinger, B. J., Lewis, P J. & Petit, B. (1979) Fetal breathing in intrauterine growth retardation. *Br J Obstet Gynaecol* **86,** 432-436.

Hytten, F E. & Leitch, I (1971) *The Physiology of Human Pregnancy* 2nd edn, Blackwell Scientific Publications, Oxford, p 26

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